The effects of elevated [CO_2] and decreased vapour pressure deficit on the nutrient status of maize and wheat plants under well-watered conditions

Kervin D. Prayag

PRYKER001

Thesis presented for the degree of Master of Science

in the Department of Biological Sciences

University of Cape Town

February 2020

Supervisors

Prof. Michael D. Cramer (University of Cape Town)

Prof. Brad S. Ripley (Rhodes University)

Plagiarism declaration

I know the meaning of plagiarism and declare that all the work in the document, save for that which is properly acknowledged, is my own. The thesis is submitted for the degree of Master of Science in the Department of Biological Sciences, University of Cape Town. It has not been for examination at any other university.

Signed: Signed by candidate Date: 10/02/2020

Abstract

Increases in photosynthetic rates (A), biomass production and grain yield have been measured across a range of C_3 plants under elevated atmospheric [CO_2] ('eCO_2'). However, decreases in the nutritional status of many C_3 plants growing at eCO_2 often occur concurrently with these increases. Several mechanisms have been proposed for these eCO_2-induced decreases, such as dilution effects due to enhanced carbohydrate production, down-regulation of photosynthesis, reduced root development, and decreased transpiration-driven mass flow delivery of nutrients. Reduced mass flow at eCO_2 is generally accepted as one cause for altered nutrient status in C_3 plants. However, eCO_2-induced reductions in mass flow remain understudied in C_4 plants, even though they account for about 18% of the total global net primary productivity and represent a large food source globally (e.g. maize and sorghum). This thesis investigated how mass flow reductions affect the nutrient status of wheat (C_3) and maize (C_4) plants. Reduced mass flow in both maize and wheat plants was induced with eCO_2 and by varying leaf-to-air vapour pressure deficits (VPD). I hypothesised that reduced mass flow at eCO_2 and at low VPD will negatively affect nutrient status in both the C_3 (wheat) and the C_4 (maize) species. In the first experiment, maize and wheat plants were grown at 400 and 800 ppm [CO_2], in three well-watered soils, ranging from sandy to clayey, with and without fertilisation. In the second experiment, plants were grown at three VPD levels, namely 1.613 kPa, 0.773 kPa and 0.350 kPa, in well-watered soil and sand. In the latter experiment, to demonstrate the importance of mass flow, plants grown in sand were supplied nutrients in such a way that they had to rely exclusively on mass flow or diffusive processes (i.e. limited interception) for nutrient delivery to their rhizosphere. eCO_2 stimulated A on average by 22% in maize and by 50% in wheat, while stomatal conductance (g_s) and cumulative water loss (CW_L) were respectively decreased by 35% and 31% in maize, and by 26% and 37% in wheat. eCO_2 reduced mass flow delivery of most nutrients on average by 32% in maize, and by 38% in wheat. The hypothesis that eCO_2-induced

reductions in mass flow negatively affect nutrient status in maize and wheat was however not supported. This was attributed to the well-watered conditions of the soils, which may have allowed for other processes (e.g. diffusion) to make up for the mass flow reductions. From 0.773 kPa to 0.350 kPa VPD, CW_L was decreased on average by 14% and 20% in the maize and wheat plants, respectively. A and g_s were little affected by VPD, but plants of both species always accumulated more biomass at 0.773 kPa. Consequently, there was little evidence to suggest that VPD-induced reductions in mass flow negatively affect nutrient status in maize and wheat. Reduced CW_L may have impeded root-to-shoot transport of ions and reduced dry biomass accumulation in the maize and wheat plants at 0.350 kPa (-40% and -22% on average respectively, relative to 0.773 kPa plants). Tissue [NPK] was also decreased (-13%, -41% and -47% respectively) in the 0.350 kPa VPD sand wheat plants, while increases in the proportion of finer roots may have alleviated effects of reduced CW_L on tissue [NPK] in the C_4 species. The findings from both experiments imply a decrease in the importance of mass flow for the delivery of nutrients to the rhizosphere under well-watered conditions. However, reductions in mass flow to a similar extent in both species at eCO_2 and low VPD — measured in the present study — suggest that under conditions of low water and nutrient availability, tissue nutrient concentrations could be negatively affected when transpiration is reduced.

Acknowledgements

I would like to first and foremost thank my main supervisor, Prof. Mike Cramer, who has provided impeccable supervision throughout my MSc. I will always strive to achieve the exemplary rigour and discipline that he applies to the scientific field. A massive thank you to Desmond Barnes, Calvan Hartnick and Dawood Hattas for their assistance in various technical aspects of my project. I cannot describe how much your contributions facilitated the execution of the growth experiments at UCT. Thank you to my co-supervisor, Prof. Brad Ripley, who has made me feel very welcome in the Botany Department at the Rhodes University since day one. Prof. Brad Ripley allowed me to use the Rhodes University Elevated CO_2 Facility, and without his collaboration, this project would not have been possible. Thank you to the technical officers (Dr. Riaan Strauss and Barry Hartley), and to the team of helpers at Rhodes University for their assistance, hard work and dedication throughout the eCO_2 experiment. Thank you to fellow students Simon Power, Kirsten Packer and Zander Venter for continuously providing valuable insights into my project. Dunja MacAlister, thank you for always being readily available to assist me with the root scans and with the IrGA. I am eternally gratefully to Olivia Venter who always motivated me throughout my Master's, assisted with lab work, and continuously provided me with moral support. Thank you, Joshua Weiss, for your assistance during harvest. A massive thanks goes to Seth Musker for his extensive proofreading and wisdom throughout the Master's. Thank you to Ian Newton (Department of Archeometry, UCT) and to Raynette Topley (Elsenburg Labs, Stellebonsch) who promptly conducted various analyses on my soil and foliar samples. I also greatly appreciated the numerous tea/coffee breaks with my fellow postgrads and lecturers, namely Anthony Lowney, Gabriella Leighton, Hana Petersen, Mohammed Kajee, Dr. Robert Thomson and A. Prof. Colin Attwood.

This project was funded by the National Research Foundation.

Table of Contents

Chapter 1

General Introduction

The global atmospheric CO_2 concentration ('[CO_2]') is increasing and is expected to reach 550 ppm by the middle of the century (IPCC, 2014). With this increase in atmospheric [CO_2], increases in photosynthetic rates, biomass production and grain yield are expected across a range of C_3 plants (Long et al., 2004; Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Leakey, 2009). These elevated [CO_2]-induced increases, however, often lead to altered nutritional status in many C_3 plants, as a result of overall decreases in concentrations of essential nutrients and minerals in the green tissue and grains of these plants. In a meta-analysis, Loladze (2014) reported between a 2% to 15% decrease (see Figure 1) in N, P, K, Ca, S, Mg, Fe, Zn and Cu and Mn in socio-economically important C_3 crops (such as rice, *Oryza sativa,* and wheat, *Tristicum aestivum*). Similarly, a 10% decline in protein levels (see Figure 2) was observed in the edible parts of 18 rice cultivars grown at elevated [CO_2] (Zhu et al., 2018). In that same study, Zhu et al. (2018) also reported up to 30% decreases in B1, B2, B5 and B9 vitamin concentrations in the grains of these rice cultivars.

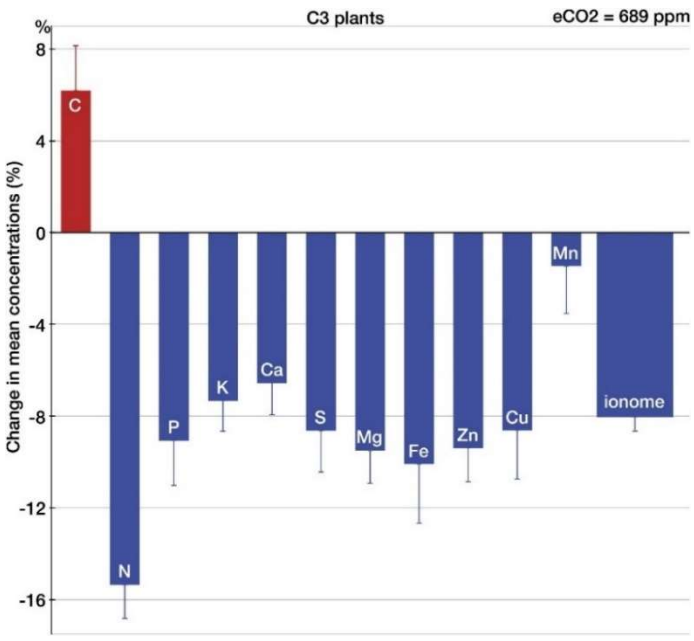


Figure 1: Changes in mean concentrations (%) of C, N, P, K, Ca, S, Mg, Fe, Zn, Cu, Mn, and in the ionome, in C_3 crop plants grown at an elevated [CO_2] concentration of 689 ppm, relative to C_3 plants grown at ambient [CO_2] (350 ppm). From Loladze (2014).

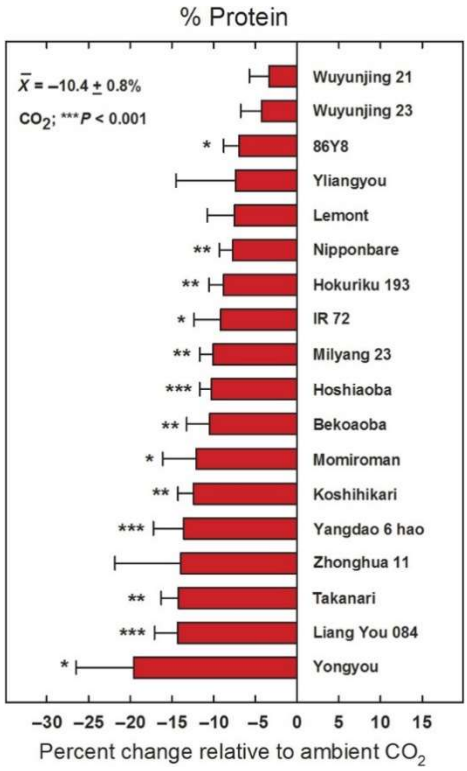


Figure 2: Changes (%) in protein concentration of 18 rice cultivars grown at elevated [CO_2] relative to ambient [CO_2]. From Zhu et al. (2018).

The decreases in essential minerals, proteins and vitamins in cereals that are socially and economically important across the globe can pose a potential threat to human nutrition (Myers et al., 2014). For example, the World Health Organization estimates that 25% of the global population suffer from Fe deficiencies, while 31% are Zn-deficient (Ezzati et al., 2004). Since crops such as wheat are also used in animal feed (in the form of grain or straw), CO_2-induced changes in nutrient status can also potentially affect the animal farming industry. For example, Porteaus et al. (2009) found that straw and grain quality in terms of crude protein was decreased in wheat plants grown at elevated [CO_2]. At an ecosystem level, the increased photosynthetic assimilation of CO_2 can lead to increased C:N ratios, with potential negative

142 impacts on nutrient cycling in natural ecosystems (Conroy, 1992). The physiological
143 mechanisms resulting in these CO_2-induced changes in plant nutrient and mineral nutrition
144 remain unclear but several hypotheses have been proposed: (1) altered root architecture and
145 physiology (2) dilution of non-carbon compounds by increased carbohydrate production from
146 enhanced photosynthesis (3) down-regulation of photosynthesis and decreased
147 photorespiration, and (4) decreased stomatal conductance and transpiration rates resulting in
148 reduced mass flow delivery of nutrients to the rhizosphere (Taub & Wang, 2008; Mcgrath &
149 Lobell, 2013).

150 Several studies have reported changes in root architecture and physiology when plants
151 are exposed to elevated [CO_2] (Berntson & Bazzaz, 1996; Pritchard & Rogers, 2000;
152 BassiriRad et al., 2001). Rogers & Runion (1994) reported an increase in root length and
153 number of root tips in crop plants correlating with high [CO_2], with the same observation being
154 made in non-cultivated systems (Berntson & Woodward, 1992; Larigauderie et al. 1994).
155 While an increase in the overall potential for nutrient and mineral uptake may be observed with
156 longer and denser root systems, exploitation and uptake efficiency may be reduced (Fitter,
157 1987; Berntson, 1994). In addition, Pritchard & Rogers (2000) experimentally demonstrated
158 that annual crops grown at elevated [CO_2] had more lateral roots versus primary roots, leading
159 to more inefficient and shallower root systems, and ultimately reducing the volume of soil
160 exploited (i.e. plants were not able to access resources in deeper soil layers). These changes in
161 root architecture and physiology therefore hinder the efficiency of below-ground uptake of
162 resources, which translates into reduced concentrations of a range of nutrients and minerals in
163 plants at elevated [CO_2].

164 Changes in the nutrient status of plants grown at elevated [CO_2] have also been
165 attributed to dilution, as a result of enhanced carbohydrate production due to increased
166 photosynthetic assimilation of C (Eng et al., 1985; Wong, 1990; Gifford et al., 2000). This

process has been experimentally demonstrated in *Chrysanthemum morifolium* grown at elevated [CO_2]. After correcting for leaf starch, Kuehny et al., (1991) found no significant differences in the concentrations of Mn, Zn and Cu between *C. morifolium* plants grown at ambient and elevated [CO_2], and therefore attributed the uncorrected concentration decreases to dilution. The effect of dilution, however, may vary depending on the ability of plants to fix N_2. For example, Taub et al. (2008) reported a 1.5% decrease in N in soybean (a N_2-fixing species), while up to a 14% decrease was observed in non-N_2-fixing species. Furthermore, growing evidence suggests that dilution may not be the main process leading to decreased nutrient and mineral concentrations in plants exposed to elevated [CO_2] (Conroy, 1992; Taub & Wang, 2008; Feng et al., 2015). Poorter et al., (1997) experimentally demonstrated that mineral concentrations across a range C_3 crops, herbaceous and woody plant species, were consistently decreased at elevated [CO_2] even after correcting for total non-structural carbohydrates. Another argument advanced against the dilution hypothesis is that in plants grown at elevated [CO_2], all the elements should experience a degree of dilution, depending on their mobility and solubility (Zhao & McGrath, 2002; Mcgrath & Lobell, 2013). However, in a meta-analysis investigating the effects of elevated [CO_2] on the concentrations of macronutrients in the edible portions of food crops, Taub & Wang, (2008) reported that N experienced a greater decrease in concentration (-12%) compared to P (-5%) and K (-4%), suggesting that other physiological processes also play a role in the observed decreases of minerals and nutrients in plants exposed to high [CO_2].

Specifically, with regards to decreases of [N] reported in plants grown at high [CO_2], physiological processes affecting plant N acquisition, rather than dilution have been suggested. One such process is the inhibition of nitrate (NO_3^-) photoassimilation, attributed to a decrease in photorespiration at elevated [CO_2] (Bloom et al., 2002; Searles & Bloom, 2003). NO_3^- reduction to NO_2^- occurs in the cytosol (Rufty et al., 1986) and requires NADH which is

192 generated from the OAA/malate and PGA/DHAP shuttles (Rathnam, 1978) that transports
193 reductant from the chloroplast (Robinson & Giersch, 1987). At elevated [CO_2], NADH
194 reductant demand for CO_2 fixation can compete with NADH for the shuttle. Furthermore, the
195 reduction of NO_2^- to NH_4^+, the assimilation of NH_4^+ into amino acids, and the Calvin cycle all
196 occur in the stroma and require ferredoxin. Elevated [CO_2] also stimulates the Calvin cycle and
197 can reduce the amount of ferredoxin available for NO_2^- reduction or NH_4^+ assimilation
198 (Baysdorfer & Robinson, 1985; Peirson & Elliott, 1988). Two studies investigating N
199 assimilation respectively in wheat and tomato plants reported a net decrease of NO_3^-
200 assimilation at elevated [CO_2] (Bloom et al., 2002; Searles & Bloom, 2003). In both studies,
201 these decreases in NO_3^- assimilation were measured by calculating the assimilatory quotient
202 (AQ), which the ratio of net CO_2 consumption to net O_2 evolution: plants assimilating less NO_3^-
203 show higher AQ values (Bloom et al., 1989). This decrease in NO_3^- photoassimilation can lead
204 to a downregulation of shoot NO_3^- and the activities of the NO_2^- reductase enzyme, which in
205 turn can potentially hinder the ability of crops to use NO_3^- as a source of nitrogen when exposed
206 to conditions of high [CO_2] (Bloom et al., 2002). Decreased NO_3^- assimilation can lead to an
207 overall decrease in plant N concentration, and negatively affect yield and quality in wheat
208 plants.

209 Another process affecting plant N acquisition at elevated [CO_2] is the decrease in the
210 activation, demand and production of the photosynthetic enzyme Rubisco (Long et al., 2004;
211 McMurtrie et al., 2008; Taub & Wang, 2008), leading to a decrease in plant N demand at the
212 leaf level. Rubisco is the most abundant leaf protein, accounting for up to 35% of total leaf N
213 in C_3 plants (Evans & Seemann, 1989; Makino, 2003). A decrease in the production of this
214 enzyme can therefore lower the overall N concentrations in plants. With this decrease in plant
215 N investment in photosynthetic and photorespiratory structures comes an increase in
216 photosynthetic plant N use efficiency (PNUE), as the N can be diverted into biomass

production (Taub & Wang, 2008). Although net losses of Rubisco have been shown in plants grown at elevated [CO_2], this is not necessarily accompanied by decreases in net photosynthetic rates (Long et al., 2004), thus the increase in PNUE in plants growing in high [CO_2]. The decrease in the investment in photosynthetic enzyme Rubisco is due to the latter being carboxylated more readily and efficiently at high [CO_2] levels (Davey et al., 1999; Stitt & Krapp, 1999; Gifford, Barrett & Lutze, 2000). When the Rubisco enzyme is saturated with CO_2, binding of the former with the latter is increased, leading to a decrease in photorespiration. In addition, in these CO_2-saturated conditions, the velocity of carboxylation by Rubisco is increased (Drake, Gonzàlez-Meler & Long, 1997). Webber, Nie & Long (1994) suggest that this situation can lead to the plants having the enzyme in excess in the photosynthetic apparatus and can represent an unnecessary investment of resources, particularly N, which could be diverted into the production of non-photosynthetic structures. However, a downregulation in the investment of photosynthetic enzymes can also decrease N availability to non-photosynthetic structures such as seeds and tubers as N is translocated to these structures from catabolised protein in the leaves (Fangmeier et al., 1999, 2002; Fangmeier, Chrost & Ho, 2000). Lower investment in the production of the Rubisco at high [CO_2] can therefore lead to reductions in overall plant N content and concentration. Furthermore, changes in N concentrations in plants can lead to cascading effects in the concentrations of other minerals and nutrients in higher plants. For example, Cakmak, Pfeiffer & McClafferty (2010) demonstrated a positive correlation between N nutrition and the uptake of Zn and Fe in durum wheat. N affects root growth and the exudation of organic compounds by the root system (Marschner, 1995; Paterson et al., 2006). Thus, adequate N nutrition can firstly increase the direct interception of Zn and Fe by the root system, and secondly can increase the mobility of these two minerals in the soil as a result of increased exudation of organic compounds. In addition to downregulation of photosynthesis, changes in NO_3^- assimilation and changes in root

242 architecture, elevated [CO_2] can lead to an overall decrease in stomatal conductance and
243 transpiration rate and can potentially represent an indirect effect of elevated [CO_2] on
244 transpiration-driven mass flow delivery of nutrients to the rhizosphere.

245 Mass flow, along with diffusion and root interception are the processes that affect
246 nutrient uptake via the root system. Uptake of nutrients by interception happens when roots are
247 directly in contact with soil particles. This process is highly dependent on specific root length
248 (root length/root mass). For example, high specific root length (SRL) leads to a high surface to
249 volume ratio. This in turn maximises the amount of root surface in contact with the soil, and
250 hence the potential of nutrient uptake (Larcher, 1995). Plants with high SRL have been found
251 to show high uptake rates of N and P (Reich, Walters & Ellsworth, 1997; Comas, Bouma &
252 Eissenstat, 2002). Diffusion acts along a concentration gradient resulting in dissolved nutrients
253 in the soil solution moving from an area of high concentration to an area of low concentration
254 until equilibrium is reached (Barber, 1995). Since there is continued uptake of nutrients by the
255 root system, nutrients continuously diffuse towards the root surface. This process is particularly
256 important in the acquisition of nutrients and minerals that are found in insoluble complexes,
257 such as Fe (Mcgrath & Lobell, 2013). In contrast to diffusion, mass flow is the movement of
258 water containing dissolved nutrients towards the roots as the plant transpires, allowing plants
259 to access nutrients from beyond their rhizosphere (Barber, 1995). Transpiration-driven mass
260 flow delivery of nutrients is considered as one of the most important processes affecting
261 nutrient and mineral uptake below ground (Russell & Barber, 1960; Chapin, 1980; Lambers et
262 al. 2008; Sellin et al., 2013). For example, Barber (1995) estimated that 79% N, 5% P, 18% K,
263 73% Ca, 88% Mg and 95% S were accumulated via transpiration-driven mass flow in maize
264 plants growing in a fertile loam soil. It is well-known that reductions in stomatal conductance
265 and transpiration rates at both the leaf and canopy scale occur when plants are exposed to
266 elevated [CO_2] (Long et al., 2004; Bernacchi et al., 2006; Ainsworth & Rogers, 2007; Leakey

et al., 2009), and this can have effects on the transpiration-driven water fluxes for delivery and transport of minerals and nutrients in plants.

Decreased mass flow delivery of nutrients to the rhizosphere at elevated [CO_2] in C_3 plants have been reported in a few studies recently. In cottonwood (*Populus deltoides*) grown at elevated [CO_2], a 20% and 40% decrease in transpiration and stomatal conductance were respectively reported, accompanied by a 20% decrease in shoot [N], which was attributed to a decrease in transpiration-driven mass flow (McDonald, Erickson & Kruger, 2002). In a meta-analysis investigating the role of transpiration and altered nutrient allocation in the changes of nutrient status,, a significant correlation between decreases in concentrations of mobile nutrients — such as K, Ca and Mg — and a decrease in mass flow at high [CO_2] was found in wheat plants grown at eCO_2 (Mcgrath & Lobell, 2013). More recently, in a free-air CO_2 enrichment (FACE) experiment, the relationship between the below-ground uptake of N, K, S, Ca, Mg and Mn and transpiration rates in wheat plants at ambient and elevated CO_2 levels was demonstrated (Houshmandfar et al., 2018). It was found that the uptake of these elements was positively correlated with transpiration rates at both ambient [CO_2] and elevated [CO_2] (average $R^2 = 0.86$). A decrease in transpiration rate at elevated [CO_2] should logically lead to a decrease in the uptake of these resources, but the data in the latter study suggested that nutrient uptake per unit of water transpired was higher at elevated [CO_2], which might suggest that the plants were able to compensate for reduced mass flow. This uncertainty about the effects of elevated [CO_2] on water fluxes and nutrient uptake in plants shows the need for more research on this topic.

The idea that mass flow is reduced at elevated [CO_2] is generally accepted as one of the causes of altered nutrient status in C_3 plants such as wheat, rice and cottonwood (Taub & Wang, 2008; Leakey et al., 2009; Mcgrath & Lobell, 2013; Myers et al., 2014). This is because of the direct link between transpiration and CO_2 uptake in these plants. In C_4 plants however, the CO_2

concentrating mechanism leads to a relative decoupling between transpiration and CO_2 intake (Conroy, 1992; Bowes, 1993; Ghannoum et al., 2000; Taiz & Zeiger, 2010; Moore & Botha, 2014). As a result, there is some uncertainty about the relative effects that high [CO_2] will have on nutrient statuses in C_4 plants (Leakey et al., 2009; Myers et al., 2014). For example, at high [CO_2], little to no effect on NO_3^- photoassimilation due to changes in photorespiration, and on Rubisco activation and requirement, should be expected in C_4 plants. Although C_4 plants account for about 18% of the total global net primary productivity (Ehleringer et al., 1997) and represent a very large food source (e.g. maize and sorghum, *Sorghum bicolor*) globally (FAOSTAT), they remain understudied in the context of mass flow acquisition of nutrients (Loladze, 2014). Furthermore, Cramer, Hoffmann & Verboom (2008) experimentally showed that a C_3 plant, *Ehrharta calycina,* was able to regulate its transpirational flux in order to improve mass flow delivery of nutrients to its root system. In contrast, Rose et al. (2018) experimentally demonstrated that C_4 grasses were unable to regulate their transpirational fluxes under varying nutrient availabilities. The authors attributed this lack of regulation to less flexible WUEs in C_4 plants. Thus, under conditions of low nutrient availability, reductions in mass flow may more severely affect the nutrient status of C_4 plants.

In a study comparing the respective responses of cotton (C_3) and maize (C_4) plants at elevated [CO_2], a comparable decrease in transpiration rates was found between the C_3 and the C_4 species, while total N and protein content was only decreased in cotton (Wong, 1979). In addition, in a meta-analysis comparing the physiological responses of C_3 and C_4 plants at elevated [CO_2], stomatal conductance was decreased to a similar degree in C_3 and C_4 plants (Ainsworth & Long, 2005). In the same meta-analysis, it was that found photosynthetic carbon assimilation in both C_3 and C_4 plants was increased at elevated [CO_2], although that increase was greater in C_3 plants. The same conclusion was reached in a literature survey, wherein CO_2 assimilation was increased in both in C_3 and C_4 plants at elevated [CO_2], with the response

being stronger in C_3 plants (Cure & Acock, 1986). On the other hand, comparable increases in net CO_2 assimilation and total plant biomass were found between wild C_3 and C_4 grass species growing at eCO_2 (Wand et al., 1999). They argued that the photosynthetic mechanism in crop species such as maize and sorghum is already saturated at ambient [CO_2] levels, whereas in the wild grasses, saturation occurs at higher [CO_2], hence the comparable effects of [CO_2] on biomass and CO_2 assimilation in these grasses. This suggests that responses observed in cultivated systems may be very different in non-cultivated systems with regards to C_4 plants. This could have important implications for ecosystems management. Although significant decreases in transpiration rate and stomatal conductance at elevated [CO_2] have been reported in C_4 plants (Wong, 1979; Ainsworth & Long, 2005), other studies have reported little to no effect of elevated [CO_2] on the nutrient status of maize in well-watered conditions (Hocking & Meyer, 1991; Conroy, 1992; Manderscheid et al., 2014). More recently, in a FACE experiment, Erbs et al. (2015) found no significant differences in concentrations of N, P, K, Ca, Mg, S, Fe and Zn in both the aboveground biomass and in the kernels of well-watered maize plants grown at elevated [CO_2], even though stomatal conductance and transpiration rate were reduced. Studies investigating the physiological responses of C_4 plants to elevated [CO_2] (such as photosynthetic rate and growth stimulation) remain scarce (Sage, 1994; Ainsworth & Long, 2005; Loladze, 2014). Furthermore, the apparent contradictory results in terms of biomass accumulation in C_4 plants at elevated [CO_2] levels between studies highlights the need for further research on this topic.

Like elevated [CO_2], low vapour pressure deficit (VPD) also leads to decreases in transpiration rates in plants. Low VPD arises when relative air humidity levels are high. Since water evaporative demand is driven by the difference of water vapour pressure between leaf tissues and the atmosphere, in low VPD conditions, evaporative demand from the atmosphere is decreased, and as a consequence, transpiration rate of plants is decreased (Lihavainen et al.,

2016). Low VPD however promotes stomatal opening which allows for CO_2 to readily diffuse into the leaf mesophyll (Bunce, 1984; Gisleröd & Nelson, 1989), which subsequently leads to increased biomass accumulation. For example, when grown at low VPD, silver birch (*Betula pendula* Roth.) trees had higher biomass and higher levels of starch but accumulated lower concentrations of N and Fe (Lihavainen et al., 2016). Experiments where plants are grown at low VPD levels represent a good way to isolate the effects of decreased transpiration on nutrient concentrations and delivery in plants. Although elevated [CO_2] also leads to decreased transpiration rates in both C_3 and C_4 plants, it also has effects of other physiological process (i.e. photorespiration, Rubisco investment, root architecture and physiology), whereas low VPD levels seem to only affect transpiration rates. The fact that elevated [CO_2] and low VPD exert differential influences on transpiration in plants potentially allows for the contributors to CO_2 depression of the ionome to be better understood.

Hypotheses

In this thesis, I evaluated the effects of elevated [CO_2] and low VPD levels on water fluxes for resource delivery to the rhizosphere, biomass accumulation, and nutrient status in a C_3 crop (wheat) and a C_4 crop (maize). I hypothesised that elevated [CO_2] levels and low VPD levels will decrease tissue nutrient concentrations in both the maize and the wheat plants as a result of reduced transpiration-driven mass flow delivery of nutrients. To test this hypothesis, we divided the thesis into two data chapters:

In Chapter 2, I investigated the extent to which mass flow delivery is reduced in wheat and maize when grown at elevated [CO_2], as a result of decreased stomatal conductance and transpiration rate. Maize and wheat plants were grown at ambient or elevated [CO_2], in five well-watered soils of differing nutrient availabilities. Accumulation of nutrients by mass flow

366 was estimated by multiplying plant water use over the whole growth period by the respective
367 concentrations of the nutrients in the soil solution. I then investigated the effects of elevated
368 [CO_2] on biomass accumulation and nutrient status in wheat and maize. I first hypothesised
369 that elevated [CO_2] will reduce tissue nutrient concentrations in wheat and maize, as a
370 consequence of reduced mass flow delivery of nutrients to the rhizosphere. I then hypothesised
371 that biomass accumulation will increase in wheat at elevated [CO_2], but not in maize, leading
372 to more pronounced decreases in nutrient concentrations in wheat plants as a result of dilution
373 effects due to the different photosynthetic pathways of the two species. Finally, I hypothesised
374 that mass flow-induced decreases in tissue nutrient concentrations in both species will be less
375 pronounced with increasing soil nutrient availability.

376 In Chapter 3, I investigated the effects of varying VPD levels on transpiration-driven
377 mass flow delivery of nutrients in maize and wheat. I expect the effects on nutrient status to be
378 comparable between the two species. As in the elevated [CO_2] experiment, accumulation of
379 nutrients by mass flow was estimated by multiplying plant water use over the whole growth
380 period by the respective concentrations of the nutrients in the soil solution. Maize and wheat
381 plants were grown in two well-watered soil treatments, a clayey soil and a sand, at three VPD
382 levels ranging from 1.613 kPa, 0.773 kPa and 0.350 kPa. In the sand treatment, to demonstrate
383 the importance of mass flow, nutrients were introduced in such a way that the roots of the maize
384 and wheat plants would not have direct access to the nutrients and would therefore have to rely
385 solely on mass flow or diffusive processes (i.e. not interception) for nutrient delivery to the
386 rhizosphere. I hypothesised that decreasing VPD levels will decrease tissue nutrient
387 concentrations in the wheat and maize plants, as a result of decreased transpiration-driven mass
388 flow delivery of nutrients to the roots. I then hypothesised that the negative effects of reduced
389 transpiration-driven mass flow on the nutrient status of the plants will be more pronounced in

390 the sand treatment, where they had to rely exclusively on mass flow and diffusion for nutrient
391 delivery to their roots.

Chapter 2

The effects of elevated atmospheric CO_2 on the nutrient status of maize and wheat under well-watered conditions

Abstract

Increases in photosynthetic rates (*A*), biomass production and grain yield are expected across a range of C_3 plants with increases in atmospheric [CO_2] ('eCO_2'). However, these increases are often accompanied by decreases in the nutritional status of many C_3 plants. Several mechanisms have been proposed for these changes, such as dilution effects due to enhanced carbohydrate production, down-regulation of photosynthesis, reduced root development, and decreased transpiration-driven mass flow delivery of nutrients. Reduced mass flow at eCO_2 is generally accepted as one cause for altered nutrient status in C_3 plants while C_4 plants remain understudied in that context even though they account for about 18% of the total global net primary productivity and represent a large food source globally (e.g. maize and sorghum). This thesis investigated how mass flow reductions affect the nutrient status of wheat (C_3) and maize (C_4) plants. Reduced mass flow was induced with eCO_2 and it was hypothesised that reduced mass flow at eCO_2 negatively affect nutrient status in both the C_3 and the C_4 species. Maize and wheat plants were grown at 400 (ambient) and 800 ppm [CO_2] ('eCO_2'), in well-watered soils of differing nutrient availabilities. eCO_2 stimulated *A* on average by 22% in maize and by 50% in wheat, while stomatal conductance (g_s) and cumulative water loss (CW_L) were respectively decreased by 35 and 31% in maize, and by 26 and 37% in wheat. eCO_2 reduced mass flow delivery of most nutrients on average by 32% in maize, and by 38% in wheat. Nutrient status, however, remained unchanged in both species, attributed to the well-watered conditions of the soils, which may have allowed for other processes (e.g. diffusion) to make up for the mass flow reductions. The findings from this study suggest that negative effects of

416 eCO_2-induced reductions in mass flow on the nutrient status of plants may potentially be
417 alleviated under well-watered conditions.

418

419 ## Introduction

420 Global atmospheric [CO_2] is on the rise and is expected to reach 550 ppm by the middle of the
421 century even under the best mitigation scenarios (Ciais et al., 2013; Smith et al., 2016). With
422 this increase in atmospheric [CO_2], increases in biomass accumulation and grain yield are
423 expected in socio-economically important crops across the globe (Long et al., 2004; Ainsworth
424 & Long, 2005; Ainsworth & Rogers, 2007; Leakey, 2009). These elevated [CO_2]-induced
425 increases, however, are often accompanied by alterations in the nutritional status in many of
426 these crops as a result of overall decreases in concentrations of nutrients essential for human
427 and animal consumption in the green tissue and edible part of these crops, such as maize and
428 wheat (Mcgrath & Lobell, 2013; Loladze, 2014).

429 Decreases in protein and mineral concentrations have been shown in wheat and maize
430 plants grown under elevated [CO_2] ('eCO_2'), both in controlled indoor environments such as
431 glasshouses and open-top chambers, as well as in experiments in open-field free-air CO_2
432 enrichment (Smith & Myers, 2019). The main mechanism proposed for these eCO_2-induced
433 changes in plant tissue and grain stoichiometry is the dilution of non-carbon compounds due
434 to increased production of carbohydrates (Loladze, 2014). For example, after correcting for
435 leaf starch, no difference was found in the concentrations of Mn, Zn and Cu between
436 *Chrysanthemum morifolium* grown at ambient and eCO_2 (Kuehny et al., 1991). Dilution effects
437 at eCO_2 may vary depending on species. Jablonski, Wang & Curtis (2002) found no significant
438 decrease in nutrient concentrations in a range of leguminous species at eCO_2 as a result of their
439 ability to fix N_2, compared to a 15% decrease in non-leguminous species. However, this

mechanism alone cannot explain the totality of the eCO_2 effects as changes in nutrient status have been observed even when biomass accumulation was not stimulated (Feng et al., 2015).

Other physiological mechanisms have therefore been proposed, such as altered root architecture and physiology in both C_3 and C_4 plant species (BassiriRad et al., 1997; Pritchard & Rogers, 2000; BassiriRad, Gutschick & Lussenhop, 2001). An increase in root length and root tips has been shown in crop plants exposed to eCO_2 (Rogers & Runion, 1994). While these changes may increase overall nutrient uptake, soil exploitation and uptake efficiency may be reduced (Berntson, 1944; Fitter, 1987). Additionally, Pritchard & Rogers (2000) showed that in crops growing at eCO_2, the ratio of lateral to primary roots increased, leading to less efficient and shallower root systems, which may then impede on the plants ability to explore the deeper layers of the soil.

Down-regulation of photosynthesis and decreased photorespiration is another mechanism proposed for altered nutrient status in plants exposed to eCO_2 (Stitt & Krapp, 1999). Downregulation of photosynthesis at eCO_2 can lead to reductions in the activity, demand for, and content of the photosynthetic enzyme Rubisco, which subsequently will decrease plant leaf N demand (Long et al., 2004; McMurtrie et al., 2008; Taub & Wang, 2008). Since Rubisco is the most abundant leaf protein and makes up to 35% of total leaf N in C_3 plants, a decrease in the production of this enzyme at eCO_2 can lead to a decrease in the total leaf N content (Evans & Seemann, 1989; Makino, 2003). In C_4 plants, such as *Sorghum bicolor*, it was found that ratios of Rubisco to PEP-Carboxylase remained constant at eCO_2 during leaf development (Cousins et al., 2003). However, in the same study, it was shown that the total activity of Rubisco was decreased in older leaves, suggesting that C_4 plants may also down-regulate photosynthetic activity at eCO_2, which can lead to reductions in leaf N demand and leaf N content.

Decreased stomatal conductance and transpiration rates at eCO_2 can also lead to reduced nutritional value of crops (Taub, Miller & Allen, 2008; Mcgrath & Lobell, 2013). These decreases can lead to reductions in overall plant water use, and subsequently in decreased transpiration-driven mass flow delivery of nutrients to the root systems of plants. Mass flow, together with diffusion and root interception are important processes affecting below-ground nutrient uptake in plants (Barber, 1995; Cramer, Hoffmann & Verboom, 2008). For example, (Barber, 1995) estimated that mass flow contributed to 79% N, 5% P, 18% K, 73% Ca, 88% Mg and 95% S in maize plants growing in a fertile loam. Cramer, Hoffmann & Verboom (2008) experimentally demonstrated that *Ehrarta calycina*, a C_3 grass, has the ability to regulate its transpirational flux in order to increase mass flow delivery of nutrients to its roots, when soil nutrient availability is low. However, this regulation was not found in C_4 grasses, wherein no increase in transpiration was measured at low soil nutrient availabilities (Rose et al., 2018). The authors attributed this lack of response to less flexible water-use efficiencies (WUE) in C_4 species. Since decreases in stomatal conductance and transpiration rate are measured in both C_3 and C_4 plants species at eCO_2 (Ainsworth & Long, 2005), the apparent inability of C_4 species to regulate transpirational fluxes at differing below-ground nutrient availabilities may put them at a greater disadvantage than C_3 plants in future climate change scenarios. Decreased mass flow of nutrients at eCO_2 is generally accepted as one of the causes of decreased nutritional value in C_3 crops such as wheat and rice (Taub & Wang, 2008; Mcgrath & Lobell, 2013; Myers et al., 2014). Due to the fact that their photosynthetic mechanism is already saturated at current CO_2 levels (Ziska & Bunce, 1997; Ainsworth & Rogers, 2007), some uncertainties remain about how eCO_2-induced decreases in mass flow will affect nutrient concentrations and content in C_4 crops such as maize (Leakey et al., 2009; Myers et al., 2014).

Often, availability of nutrients and water interact with and dictate the effects of eCO_2 on plant biomass and nutrient status (Körner, 2006). For example, it has been argued that

489 negative effects of eCO_2 on grain protein concentration in wheat would be alleviated with
490 increased availability of N (Evans, 1993; Pleijel et al., 1999). However, in a more recent study,
491 it was shown that grain protein concentration in this C_3 crop significantly decreased at eCO_2
492 regardless of N fertilisation (Pleijel et al., 2019). Additionally, it also been shown that
493 downregulation of photosynthesis in C_3 plants happens to a greater extent in low N
494 environments compared to high N environments (Stitt & Krapp, 1999; Ainsworth & Rogers,
495 2007). In maize grown at eCO_2, it was found that yield and leaf physiological capacity
496 remained unchanged across a range of soil N fertility, provided the plants were not water-
497 stressed (Markelz, Strellner & Leakey, 2011). In the same study, decreases in leaf number, leaf
498 area index, biomass, and yield were recorded at low N availability in the maize plants grown
499 at both ambient and eCO_2, suggesting that nutrient stoichiometry was not affected by variation
500 in atmospheric [CO_2].

501 With regards to interactions between soil water availability and eCO_2, it was found that
502 stomatal conductance decreased by 51% in droughted wheat plants grown at eCO_2, compared
503 to a 41% decrease under well-watered conditions (Li, Kang & Zhang, 2004). Similarly, in
504 maize plants, stomatal conductance at eCO_2 was reduced by 57% in droughted conditions, and
505 by 33% in well-watered conditions (Markelz, Strellner & Leakey, 2011). Since mass flow is
506 directly related to plant transpiration, differing watering regimes are likely to influence eCO_2-
507 induced decreases in mass flow delivery of nutrients, and subsequently influence eCO_2 effects
508 on nutrient status. Increased water supply may thus alleviate the negative effects of eCO_2-
509 induced decreases in mass flow on nutrient status, especially in C_4 plants with less flexible
510 WUEs than C_3 plants (Ainsworth & Rogers, 2007). While there are some similarities as to how
511 eCO_2 affects stomatal conductance and transpiration rates in C_3 and C_4 plants, the eCO_2 effects
512 on photosynthesis differ (Ainsworth & Rogers, 2007). Stimulation of photosynthesis in C_3
513 crops grown at eCO_2 seem to occur under a range of watering regimes (Kimball, 2016). In

maize plants however, stimulation of photosynthesis at eCO_2, and subsequent increase in biomass, was generally found only under dry conditions, as a result of improved water-use efficiency (Leakey et al., 2009; Markelz, Strellner & Leakey, 2011; Erbs et al., 2014). Erbs et al. (2014) argue that eCO_2 could mitigate the impacts of drought stress on nutritional quality of maize plants. However, increases in biomass accumulation, combined with reductions in stomatal conductance (and consequently reductions in mass flow delivery of nutrients), could severely decrease the nutritional status of these C_4 plants under conditions of drought and eCO_2.

Since decreases in stomatal conductance and transpiration rates have been reported in both maize and wheat at eCO_2 (Ainsworth & Rogers, 2007; Leakey et al., 2009), I hypothesised that these reductions will lead to a decrease in tissue nutrient concentrations in both the C_3 and the C_4 crop as a result of reduced mass flow delivery of nutrients. I then hypothesised that reduced root development at eCO_2 in both species will also contribute to decreases in tissue nutrient concentrations. Since photosynthesis is mostly likely to be stimulated by eCO_2 in plant C_3 species compared to C_4 plant species under well-watered conditions (Ainsworth & Rogers, 2007), I hypothesised that nutrient concentrations will be decreased to a greater extent in wheat compared to maize, as a result of dilution effects due to enhanced carbohydrate production. Finally, I hypothesised that the negative effects of eCO_2-induced reductions in mass flow on the nutrient status of both crop species will be lessened with increasing soil nutrient availability (Körner, 2006; Mcgrath & Lobell, 2013). To test these hypotheses, maize and wheat plants were grown at two CO_2 levels, under well-watered conditions, and in soils with differing nutrient availabilities, and changes in nutrient concentrations and contents were measured for several important nutrients.

Methods

Plant germination and growth conditions

Wheat (*Triticum aestivum* c. SST015, Kaap Agri, Malmesbury, 7300, South Africa) and maize (*Zea mays* c. CAP 122-60, Capstone Seeds, Howick, 3290, South Africa) were grown from seeds soaked in aerated water for 24 h and 48 h, respectively, before being sown in trays of vermiculite. The trays were kept moist until the emerging seedlings were established. The seedlings were transplanted into pots, each containing 7 kgs of soil. Three soils of varying clay content were used in this experiment: a top soil with high clay content (hereafter referred to as 'Top Soil'), sand with low clay content (hereafter referred to as 'Sand'), and a mix of sand (70% by mass) and top soil (30%), with moderate clay content (hereafter referred to as 'MIX').

Plants growing in Top Soil and MIX were subjected to two fertilisation treatments: an unfertilised treatment and a treatment fertilised with 25 g of Protek (Farmwox (Pty) Ltd Reg. No.: B4652) pellets containing (mmol.g^{-1}) 3.43 N; 0.32 P; 0.92 K; 0.0003 Cu; 0.016 Fe; 0.004 Mn and 0.00009 Zn. The fertilised treatments of Top Soil and MIX are hereafter respectively referred to as 'Top Soil NPK' and 'MIX NPK'. Plants growing in Sand were always subject to fertilisation, with 25 g of Protek pellets and a modified Long-Ashton solution containing only 1.5 mM $MgSO_4.7H_2O$, 4 mM $CaCl_2.2H_2O$, 0.1388 mM H_3BO_3, 0.0208 mM $MnSO_4.4H_2O$, 0.0023 mM $ZnSO_4.7H_2O$, 0.0033 mM $CuSO_4.5H_2O$, 0.0002 mM $Na_2MoO_4.2HO$ and 0.0899 Fe EDTA (Hewitt, 1966). The Sand pots were first supplied with excess water before being supplied with 200 mL of solution twice weekly. The soil fertilisation treatments (hereafter referred to as 'soil treatments') were chosen so as to have a nutrient availability gradient. Thus 'Top Soil', 'Top Soil NPK', 'MIX', 'MIX NPK' and 'Sand' were treated as distinct soil treatments with different nutrient availabilities (see Table 1 below).

These soil treatments were replicated so as to have wheat and maize plants subject to them at either 400 ppm (hereafter referred to as 'ambient'), or at 800 ppm (hereafter referred to as 'eCO_2'). The plants growing at ambient CO_2 were randomly distributed between two open-top chambers into which the atmospheric [CO_2] was set at 400 ppm. Similarly, the plants growing at eCO_2 were randomly distributed between two open-top chambers into which the atmospheric [CO_2] was set at 800 ppm. The experiment was thus conducted using a split-plot experimental design, with the four open-top chambers being the whole-plots, and the split-plots being the different soil treatments in which the plants were growing. The experiment was conducted at the Rhodes University Elevated CO_2 Facility (Rhodes University, Grahamstown, South Africa, 33°18'41.2"S 26°30'33.4"E).

Field capacity and water use

Prior to running the experiment, the respective field capacities of the three soil types were determined. This was done by supplying excess water to pots containing 200 g of soil that had been oven-dried 7 d at 70°C to remove any moisture. The pots were covered with plastic sheets to prevent evaporation and were allowed to settle for 24 h. Field capacity of the different soil types was then determined as the dry weight percentage of the difference between the dry weight of the soil and the wet weight 48 h after they had settled (Kirkham, 2005). The field capacities were 28%, 17% and 14 % for Top Soil, MIX and Sand, respectively. Throughout the experiment, the soils were kept between 70-100 % of their respective field capacities by gravimetric measurement of water content. These field capacities were chosen to allow unrestricted mass flow (Marschner & Rengel, 2011). For each soil type, 10 additional control pots filled with the same amount of soil (7 kg) were randomly distributed in the two open-top chambers set at ambient, and in the two open-top chambers set at eCO_2. These control pots were subject to the same watering regime as the bags with the plants. This was done so as to gravimetrically estimate the cumulative water use per plant throughout the growth experiment.

585 *Soil nutrient availability (shown in Table 1)*

586 A soil column was constructed from a 20-mL syringe with the plunger removed, and a disc of
587 Whatman No. 1 filter paper was placed at the bottom. The filter paper was topped with glass
588 wool to prevent loss of sediment. Oven-dried soil was filled to the 20 mL mark and water added
589 to field capacity. The soil column was inserted through a hole in the lid of a 50-mL Falcon
590 centrifuge tube, placed in the tube, and centrifuged (Eppendorf Centrifuge 5810R) at 150 g for
591 2 min. The volume of water collected in the centrifuge tube was taken as a relative measure of
592 the mass flow displaceable soil solution. The mass flow technique using low-speed
593 centrifugation was a slight modification of the gravity method described by (Barber, 1995).
594 The elemental determination in the water extracts were performed by the Elsenburg Laboratory
595 (Western Cape Department of Agriculture, Stellenbosch, South Africa, 33.845259 S;
596 18.834722 E). The concentrations of P, K, Ca, Cu, Fe, Mn and Zn were determined directly
597 using a Radial ICP Spectrometer ICAP 7600 (Thermo Fisher Scientific, Sunnyvale, CA, USA),
598 while N was determined on a BR-71394 DA Gallery Analyzer (Thermo Fisher Scientific,
599 Sunnyvale, CA, USA). Concentration of most nutrients in water extracts from Top Soil NPK,
600 MIX NPK and Sand were generally higher compared to water extracts from Top Soil and MIX
601 (Table 1).

602

603 **Table 1:** Concentrations (mg l^{-1}) of N, P, K, Ca, Cu, Fe, Mn and Zn in soil solution extracted
604 from the five soil types in which the wheat and maize plants were grown. Different lower case
605 letters indicate the significant differences amongst soil treatments as determined by Tukey's
606 post-hoc comparisons.

	Top Soil	MIX	Top Soil NPK	MIX NPK	Sand
Total inorganic N	10.68 (a)	9.29 (a)	32.63 (b)	46.57 (c)	49.04 (c)
Phosphorus	1.00 (a)	1.00 (a)	4.20 (b)	6.80 (c)	14.00 (d)
Potassium	17.80 (a)	14.80 (a)	29.00 (b)	37.00 (c)	42.40 (d)
Calcium	78.00 (a)	68.20 (a)	107.00 (b)	117.80 (b)	128.80 (b)
Copper	0.05 (a)	0.04 (a)	0.05 (a)	0.06 (a)	0.07 (a)
Iron	2.60 (d)	1.46 (bc)	1.68 (c)	0.88 (ab)	0.65 (a)
Manganese	0.04 (a)	0.05 (a)	0.06 (b)	0.09 (c)	0.09 (c)
Zinc	0.87 (b)	0.61 (b)	0.87 (b)	1.58 (c)	0.16 (a)

607

608 *Gas exchange measurements*

609 All plant gas exchange measurements were conducted on fully expanded leaves using a LI-
610 6400 (Li-Cor, Lincoln, NE, USA) Portable Photosynthesis System equipped with 2 x 3 mm
611 broadleaf cuvette and an integrated red/blue LI-6400-02B LED light source (Li-Cor, Lincoln,
612 NE, USA). I set the [CO_2] in the cuvette to 400 or 800 ppm depending on the CO_2 level at
613 which the plants being measured were exposed. Relative humidity was set to between 55-65%
614 and block temperature was set to match the temperature of the day of which the measurements
615 were being taken. The variables measured were photosynthetic rate, stomatal conductance and
616 transpiration rate.

Plant harvest

The wheat and maize plants were harvested after 7 weeks of growth. The soil was carefully washed away with water on a 2-mm sieve to minimise root loss. The plants were separated into root, stem and leaves, the surface dried with paper towel, and immediately weighed to obtain fresh weight (stem and leaves were later combined to obtain above-ground biomass). The leaves were then scanned with a flatbed scanner (Canon CanoScan LiDE 220) so as to obtain the leaf area and thus the specific leaf area (SLA) of each plant. The harvested plants were then oven-dried at 70°C for 48 h after which dry weight was determined. A representative sub-sample of the root system of each plant (Vandamme et al., 2013) was used to determine average root diameter (mm) and total root surface area (m^2) using a STD4800 scanner and WinRHIZO version 2013a (Regent Instruments, Canada). Specific root length (SRL, m g^{-1}) was calculated as the total root length (m) divided by the root dry weight (g). The root characteristics were converted to whole-root results by multiplying by their respective conversion factors to 100% of the root.

Mass flow delivery and contribution

Mass flow delivery of each nutrient was calculated as the cumulative amount of water transpired by a plant throughout the growth experiment multiplied by the concentration of that nutrient in the soil solution. Since delivery of a nutrient does not necessarily equate to acquisition, mass flow contribution to tissue concentration of that nutrient was then calculated as a percentage of the total concentration of that nutrient in the plant. The rest was assumed to have been acquired by diffusion and/or root interception (Barber, 1995).

639 *Foliar elemental analyses*

640 The dried foliar and root samples were ground to a fine powder using a grinder or a ball-mill
641 (MM200, Retsch, Germany). The powdered samples were placed in Perspex rings sealed with
642 4 μm Polypropylene thin film (Chemplex Industries Inc, Florida, USA) and introduced to a
643 SPECTRO XEPOS XRF spectrometer (SPECTRO, AMATEK materials analysis division,
644 Kleve, Germany). Analyses were conducted using the X-LabPro 5 software, which
645 incorporates the universal 'Turbo Quant Powders' method. The instrument was calibrated by
646 using a certified standard GBW07312 (National Research Center for CRMs, Beijing, China),
647 for which elemental concentrations were obtained from NOAA Technical memorandum NOS
648 ORCA 68 (1992). Only the elements that were within the machine's detection limits were
649 included.

650 Total N concentration of the powdered foliar and root samples were determined using
651 mass spectrometry. Samples were weighed into tin capsules (5 x 9 mm; Säntis Analytical,
652 Teufen, Switzerland, with *ca*. 2 mg of foliar powder used for analysis). The samples were
653 combusted in a Flash 2000 organic elemental analyser and the gases passed into a Delta V Plus
654 isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (all from Thermo
655 Scientific, Bremen, Germany). In-house standards and one IAEA (International Atomic Energy
656 Agency) standard (USGS25) were used to calibrate the results. Nitrogen concentration was
657 expressed relative to atmospheric nitrogen (Evans, 2001). Ratios of the different elements were
658 obtained by dividing their respective concentrations by their carbon concentrations.

659 *Data analyses*

660 All statistical analyses were performed using R Statistical Software (R Core Team, 2019).
661 Comparisons of all the response variables were made using linear models with CO_2 level and
662 soil treatment as explanatory variables, fitted as interactions, or as separate main effects if the

663 interaction was not significant. Random effects were included in the fitted models to account
664 for the non-independent structure of the experimental design, since plants growing in one
665 open-top chamber are more likely to experience similar growing conditions compared to
666 those growing in another open-top chamber – that is even if they were growing in the same
667 soil treatment and were exposed to the same CO_2 concentration in two different open-top
668 chambers. However, the random effects were found to be non-significant in all instances,
669 potentially demonstrating the homogeneity in terms of the controlled environment that the
670 open-top chambers at the Rhodes University Elevated CO_2 Facility can provide.
671 Consequently, the random effects were dropped from the models, and the data was analysed
672 in bulk (in terms of chambers). The concentrations of N, P, K, Ca, Cu, Mn, Fe and Zn in the
673 water extracts from the different soil treatments were also compared using linear models,
674 with concentrations of the elements as the response variables, and soil treatment fitted as the
675 explanatory variable. Analyses of variance were done on all the fitted linear models to
676 determine the significance of the main effects using the *'car'* package (Fox & Weisberg,
677 2011). Where main effects significantly explained variation, pairwise comparisons (Tukey
678 post-hoc tests) of group level means were then performed using the package *'emmeans'*
679 (Lenth, 2018) to determine which soil treatments showed a significant effect of [CO_2]. To test
680 the relationship between mass flow delivery and nutrient concentration, the data were pooled
681 in terms of soil treatments to fit linear models with tissue nutrient content as the response
682 variable and mass flow delivery of nutrients as the explanatory variable. The same was done
683 to generate correlation plots between these two variables. Boxplots and correlation plots were
684 all generated using the *'ggplot2'* package (Wickham, 2016).

685 Figures 1 to 10 and Appendices 1 to 12 show the effects of soil treatment and [CO_2] on
686 morphological, physiological and nutrient variables in the maize and wheat plants. The
687 ANOVA results indicating the significance of each factor are shown in each plot, with

interaction terms excluded if they were not significant ($p > 0.05$). Note that a significant interaction term implies that the effect of [CO_2] varied between soil treatments.

Results

Total biomass, root biomass, shoot-to-root ratio and root characteristics

eCO_2 did not have any significant effect on total biomass, root biomass or shoot-to-root ratio in maize plants (Fig. 1). eCO_2 increased total biomass and root biomass in wheat plants, but this was dependent on soil treatments. Total biomass was significantly higher in wheat plants at eCO_2 in MIX NPK, and there was no difference in tissue nutrient concentrations in both species exposed to the two CO_2 levels in the other soil treatments. Root biomass was higher at eCO_2 in MIX NPK and Top Soil NPK, with no difference between the two CO_2 levels in the other soil treatments. There was no difference in shoot-to-root ratio in wheat between the two CO_2 levels in any of the soil treatments.

There was no effect of eCO_2 on average root diameter, total surface area and specific root length in maize plants within each soil treatment (Fig. 2). Wheat plants grown at eCO_2 showed increased average root diameter in all soil treatments, but no differences in total surface area. Wheat plants grown at eCO_2 generally showed decreased specific root length, but this effect was dependent on soil treatment, with the only significant reduction occurring in plants grown in MIX NPK.

Gas exchange and water use

Stomatal conductance and transpiration rate were decreased in both maize and wheat plants grown at eCO_2 in all soil treatments (Fig. 3). eCO_2 increased photosynthetic rate in wheat plants in all soil treatments. There was an overall significant effect of [CO_2] on photosynthetic rate in

maize plants, but post-hoc results showed no difference between the two [CO_2] levels within each soil type. Cumulative water use (i.e. total water loss) per plant was decreased in maize plants grown at eCO_2 in all soil treatments (Fig. 4). This was not the case in wheat where cumulative water use per plant was decreased in Top Soil, MIX and Sand, but not in Top Soil NPK and MIX NPK.

Mass flow delivery and contribution

The eCO_2-induced reduction in cumulative water use resulted in decreased mass flow delivery of N, P, K, Ca, Cu, Mn, Fe and Zn in maize plants in all soil treatments (Fig. 5, Appendices 1 and 2). Similarly, in wheat, eCO_2 plants had reduced delivery of all the nutrients by mass flow, but this was dependent on soil treatment, since in Top Soil NPK and MIX NPK, mass flow delivery was not reduced. Maize plants grown at eCO_2 also had decreased mass flow contribution to tissue content of most nutrients (except Fe), but this was dependent on soil treatment with the only significant reduction occurring in plants grown in Sand (Fig. 6, Appendices 3 and 4). In wheat, eCO_2 plants showed significantly reduced mass flow contribution of most nutrients in all soil treatments.

Nutrient concentrations, contents, ratios and, tissue nutrient content versus mass flow delivery

Despite marked changes in variables relating to nutrient uptake, there were no differences in tissue concentrations of most of the nutrients in wheat and maize plants grown at the two CO_2 levels (Fig. 7, Appendices 5 and 6). Tissue nutrient contents were also little affected by eCO_2 in maize (Fig. 8, Appendices 7 and 8). In wheat, contents of all the elements, except for Fe, were increased at eCO_2 in the MIX NPK soil. Additionally, contents of N, Ca, Mn and Fe were increased at eCO_2 in the Top Soil NPK treatment.

733 Linear regressions revealed significant positive correlations between N, P and K tissue
734 content and amount of these nutrients delivered by mass flow, in both maize and wheat (Fig.
735 9). Additionally, the intercepts (i.e. when 0 mg N, P, and K delivered by mass flow) were higher
736 at eCO_2 in both maize and wheat for N, P and K. The correlation between amount of Fe
737 delivered by mass flow and tissue Fe content revealed a significant negative correlative in
738 wheat plants at both ambient and eCO_2 (Appendix 10). For the other nutrients, depending on
739 the species, correlations were either not significant at ambient, eCO_2 or both [CO_2] levels
740 (Appendices 9 and 10).

741 Wheat plants grown at eCO_2 showed increased C:N and C:P ratios in all soil treatments
742 except MIX (Fig. 10). Increased C:K, C:Ca, C:Mn and C:Zn ratios were observed in wheat
743 plants grown in the MIX NPK soil (Fig. 10, Appendices 11 and 12). Changes in these ratios
744 can indicate dilution effects of some elements as a result of enhanced carbohydrate production
745 at eCO_2. In contrast to wheat, maize plants grown at eCO_2 did not have different ratios.

746

747 ## Discussion

748 In agreement with the literature, eCO_2 reduced stomatal conductance and transpiration rates in
749 both maize and wheat plants (Long et al., 2004; Bernacchi et al., 2006; Ainsworth & Rogers,
750 2007; Leakey, 2009). Cumulative water use per plant was also decreased in both species at
751 eCO_2, except for wheat plants growing in Top Soil NPK and MIX NPK. As such, transpiration-
752 driven mass flow delivery of nutrients was significantly decreased at eCO_2, except in the wheat
753 plants growing in Top Soil NPK and MIX NPK. However, there was little to no decrease in
754 tissue nutrient concentrations at eCO_2 in either of the crops, regardless of soil treatment. Since
755 no decrease in mass flow delivery of nutrients was recorded in the wheat plants growing in Top
756 Soil NPK and MIX NPK, adequate supply of nutrients can explain the lack of change in their

757 tissue nutrient concentration at eCO_2. The following question therefore arises: why then were
758 there no decreases in tissue nutrient concentrations in both maize and wheat at eCO_2 even in
759 the instances where mass flow delivery of nutrients was significantly decreased?

760 The amount of a nutrient displaced by mass flow is strongly dependent on its solubility
761 in soil water (Barber, 1995). For example, Ca is highly soluble and is often delivered in excess
762 to the rhizosphere (Lambers, Chapin & Pons, 1998; Zhao & McGrath, 2002; Cramer &
763 Hawkins, 2009). Fe complexes are generally less soluble, making the diffusive process more
764 important than mass flow for the transport of these nutrients (Mcgrath & Lobell, 2013).
765 Additionally, it is important to make the distinction between nutrient delivery and acquisition,
766 whereby delivery by mass flow does not necessarily equal acquisition. In agreement with the
767 literature (Cramer & Hawkins, 2009), it was found that Ca was delivered in excess of
768 physiological requirements at both ambient and eCO_2 in both maize and wheat plants. As such,
769 the decreases in mass flow contribution of Ca measured at eCO_2 in both the C_3 and C_4 crop
770 species in the present study did not negatively impact their tissue Ca concentration or content.
771 Furthermore, negative relationships (and non-significant in maize) were found between tissue
772 Fe content and mass flow delivered Fe in both species, suggesting that diffusive processes or
773 root interception may have been more important for the delivery of this nutrient to the
774 rhizosphere. Thus, even though a decrease in mass flow contribution of Fe was measured in
775 some soil treatments in the wheat plants at eCO_2 (no decrease in maize), no decrease was
776 measured in the concentration of this nutrient in either of the species.

777 Mass flow and diffusive processes are strongly dependent on water flow in the soil
778 (Barber & Cushman, 1981; Brouder & Volenec, 2008), and so is their relative importance
779 (Cramer, Hoffmann & Verboom, 2008). Thus, the well-watered conditions (between 70-100%
780 field capacity) to which the maize and wheat plants were subject, at both ambient and eCO_2,
781 may have allowed for these two processes to adequately and interchangeably transport nutrients

782 to the root systems, regardless of soil nutrient availability, even though cumulative water use
783 per plant and mass flow contribution of most of the elements were reduced at eCO_2. Schmidt
784 et al (2004) showed that although an increase in the soil solution concentrations of several
785 elements can occur during and after a drought event, the flux of these elements is generally
786 lower due to decreased water flow in water-stressed environments. The well-watered status of
787 all the soil treatments in the present study can therefore explain the lack of negative effects of
788 eCO_2-induced reductions in mass flow delivery of nutrients on the nutrient status of the plants.
789 Additionally, eCO_2 tends to significantly affect mass flow while having small effects on
790 diffusive processes (Mcgrath & Lobell, 2013). Therefore, reduced mass flow induced by eCO_2,
791 if combined with conditions of water stress, could lead to a decrease in the tissue nutrient
792 concentrations of these two crops. When looking at the relationship between mass flow
793 delivery and tissue contents of N, P and K, the significantly higher contents of N, P and K at
794 eCO_2 in the absence of mass flow (at intercept) in both species, suggests a decrease in the
795 importance of this process, in favour of other processes such as diffusion or interception for
796 the delivery and acquisition of these nutrients. These results may explain the patterns reported
797 in the study by Kimball (2006), in which decreases in tissue nutrient concentrations in wheat
798 and cotton grown at eCO_2 were alleviated in well-watered treatments (4% decrease in foliar N
799 concentration as opposed to 9% under limited water supply). Thus, the hypothesis that eCO_2-
800 induced decreases in mass flow delivery of nutrients negatively affect the nutritional value of
801 maize and wheat is not verified in this study, possibly as a result of the well-watered conditions
802 of all the soil treatments.

803 When considering the altered root architecture hypothesis, the lack of changes in
804 specific root length and total surface area in both maize and wheat at eCO_2, in addition to
805 constant flow of water below-ground, may have allowed for adequate nutrient delivery and
806 uptake, and can further explain the lack of difference in tissue nutrient concentrations between

807 the two CO_2 levels in both species. With regards to root diameter, BassiriRad et al. (1997)
808 demonstrated that C_4 plants were able to increase the proportion of finer roots and maintain
809 adequate N and P concentrations at eCO_2, while the concentrations of these nutrients were
810 decreased in C_3 plants. Additionally, Gillespie (1989) has demonstrated that finer roots can
811 more efficiently extract nutrients such as N, P and K from the soil. In the present study, root
812 diameter was increased in the wheat plants grown at eCO_2, thereby potentially leading to less
813 efficient root systems for belowground nutrient uptake in the C_3 crop (Luo et al., 2013).
814 Nonetheless, nutrient concentrations remained unchanged in both the C_3 and the C_4 species. In
815 a recent study done by Houshmandfar et al. (2018), it was shown that eCO_2 can increase the
816 nutrient uptake per unit of water transpired in wheat, attributed to increased root growth in the
817 earlier stages of growth. Data on root growth at different stages of growth were not available
818 in the present study, but it is possible that changes in root ontogeny allowed the eCO_2 wheat
819 plants to maintain a similar nutrient uptake rate to that of their ambient CO_2 counterparts.
820 Therefore, the hypothesis that altered root architecture at eCO_2 will lead to decreases in
821 nutritional value of the C_3 and C_4 crop is also not verified in this study.

822 eCO_2 significantly increased photosynthetic rate in wheat within each soil treatment,
823 but this was not observed in maize, which is expected due to the differing C_3 and C_4
824 photosynthetic pathways. The photosynthetic mechanism of C_4 plants is generally saturated at
825 the current ambient atmospheric CO_2 (Ziska & Bunce, 1997), although there is evidence for
826 stimulation of photosynthesis at eCO_2 even in C_4 plants (Leakey et al., 2004; Long et al., 2004).
827 Since there was no evidence of downregulation of photosynthesis in the maize or wheat plants
828 at eCO_2, it is possible that photosynthetic demand for N was similar, or increased (i.e. in the
829 wheat plants) relative to the ambient CO_2 plants, thus leading to little or no difference in tissue
830 N concentration between the two CO_2 levels. This study therefore does not provide evidence
831 of decreased nutritional value in maize or wheat as a result of downregulation of photosynthesis

832 at eCO_2. In the wheat plants growing in the MIX NPK soil treatment, eCO_2 significantly
833 increased biomass accumulation. This is most likely because the stimulation of photosynthesis
834 at eCO_2 in these plants interacted with the adequate supply of nutrient and water in this soil
835 treatment. However, a significant increase in the C:N and C:P ratios was measured in these
836 plants, providing some evidence of a dilution effect of these two elements (Taub & Wang,
837 2008). Although eCO_2, combined with high nutrient availability and adequate water supply,
838 increased biomass accumulation and nutrient content in the MIX NPK wheat plants, this
839 positive effect seemed to have been cancelled out by the dilution of some elements. In contrast
840 to wheat, photosynthetic rate and biomass accumulation of maize plants were not affected at
841 eCO_2 under well-watered conditions, results which are in agreement with the literature
842 (Ainsworth & Rogers, 2007; Markelz, Strellner & Leakey, 2011). Additionally, no evidence of
843 dilution of any of the elements was found in the C_4 crop. However, in drought conditions at
844 eCO_2, maize plants are able to improve WUE as a result of decreased stomatal conductance
845 and sustained intercellular [CO_2], which can subsequently lead to increased biomass
846 accumulation (Allen et al., 2011). I therefore suggest that increased biomass accumulation in
847 maize plants under drought conditions, combined with reduced mass flow delivery of nutrients
848 at eCO_2, can potentially decrease tissue nutrient concentration in maize plants as a result of
849 dilution effects. However, this effect was not seen in the maize plants in this study possibly
850 because they were not water-stressed.

851

852 ## Conclusion

853 The results from the experiment strongly suggest that the well-watered status of the soils may
854 have allowed for an increase in the importance of diffusive processes for nutrient delivery to
855 the rhizosphere when the plants were exposed to eCO_2, thereby making up for the reductions

856 in mass flow. As such, no decrease in tissue nutrient concentration was measured in the maize
857 or wheat plants grown at eCO_2. These findings thus highlight the importance of considering
858 soil water status when investigating and interpreting the effects of eCO_2-induced decreases in
859 mass flow on the nutrient status of plants. Growth at eCO_2 under conditions of limited water
860 availability, conditions which are not unlikely according to future climate predictions (Gerten
861 et al., 2004; Fisher, Nakicenovic & Alfsen, 2007; Mu et al., 2007), may potentially negatively
862 impact the quality and yield of important crops globally, as a result of decreased mass flow,
863 and subsequently inadequate supply of nutrients.

864

865

866 ## List of figures

867 **Figure 1:** Variation in total biomass, root biomass and shoot-to-root ratio in maize and wheat
868 plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800
869 ppm. The boxes and horizontal lines represent the first and the third quartiles and the medians,
870 respectively. The whiskers represent 1.5 × the interquartile range and outliers above/below are
871 shown as points. The diamonds represent the mean values and the filled circles indicate
872 outliers. The F- and p-values of the main effects and interaction (when significant) from the
873 analysis of variance done on the linear models fitted are displayed. Lower case letters indicate
874 the significant differences between treatments as determined by Tukey's post-hoc
875 comparisons.

876 **Figure 2:** Variation in average root diameter, total surface area and specific root length in
877 maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2
878 levels, 400 and 800 ppm. Statistics as in Figure 1.

Figure 3: Variation in stomatal conductance, transpiration rate, and photosynthetic rate in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Figure 1.

Figure 4: Variation in cumulative water loss measured by gravimetry in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Figure 1.

Figure 5: Variation in transpiration-driven mass flow delivery of N, P and K in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Figure 1.

Figure 6: Variation in transpiration-driven mass flow contribution of N, P and K to tissue content in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. The dashed line represents 100% contribution to tissue content. Statistics as in Figure 1.

Figure 7: Variation in total tissue concentrations of N, P and K in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Figure 1.

Figure 8: Variation in total tissue contents of N, P and K in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Figure 1.

Figure 9: The relationship between transpiration-driven mass flow delivery of N, P and K, and total tissues contents of N, P and K, in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. The intercept, slope, R^2 and p-values of the lines are displayed, as well as the p-value of the contrast between the 400 and 800 ppm line.

903 **Figure 10:** Variation in the C:N, C:P and C:K ratios in maize and wheat plants, grown in five
904 soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in
905 Figure 1.

906

Figures

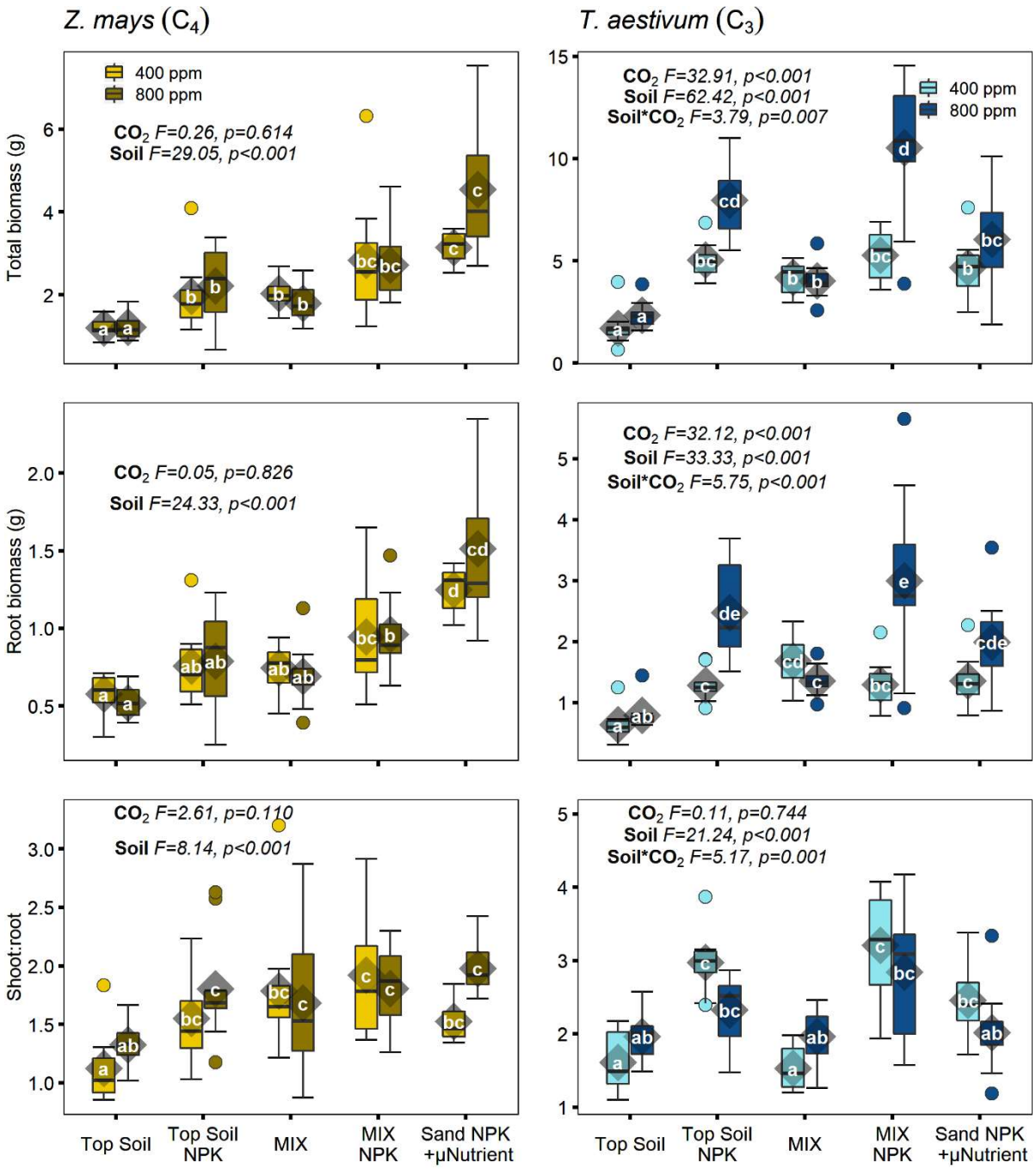
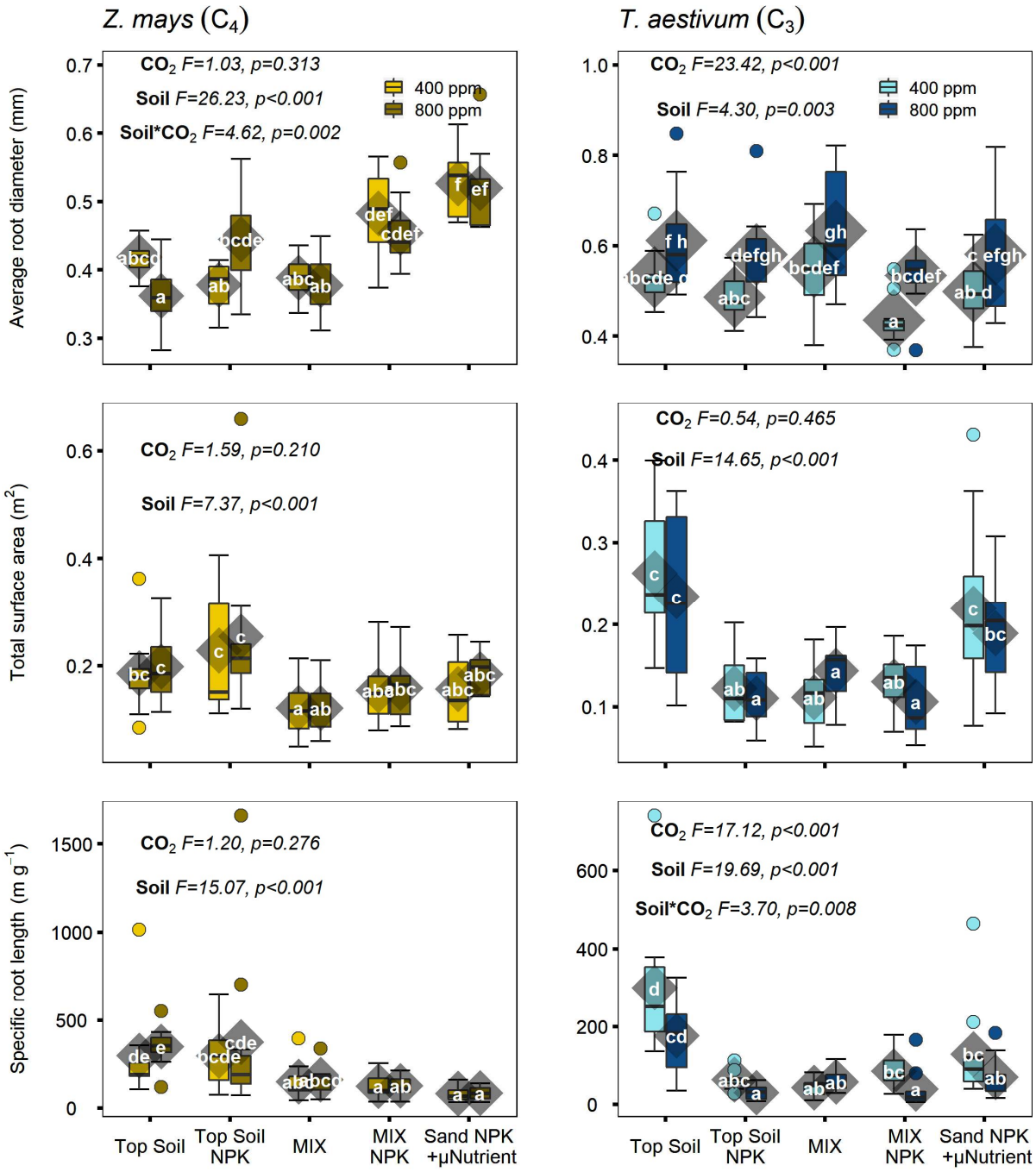


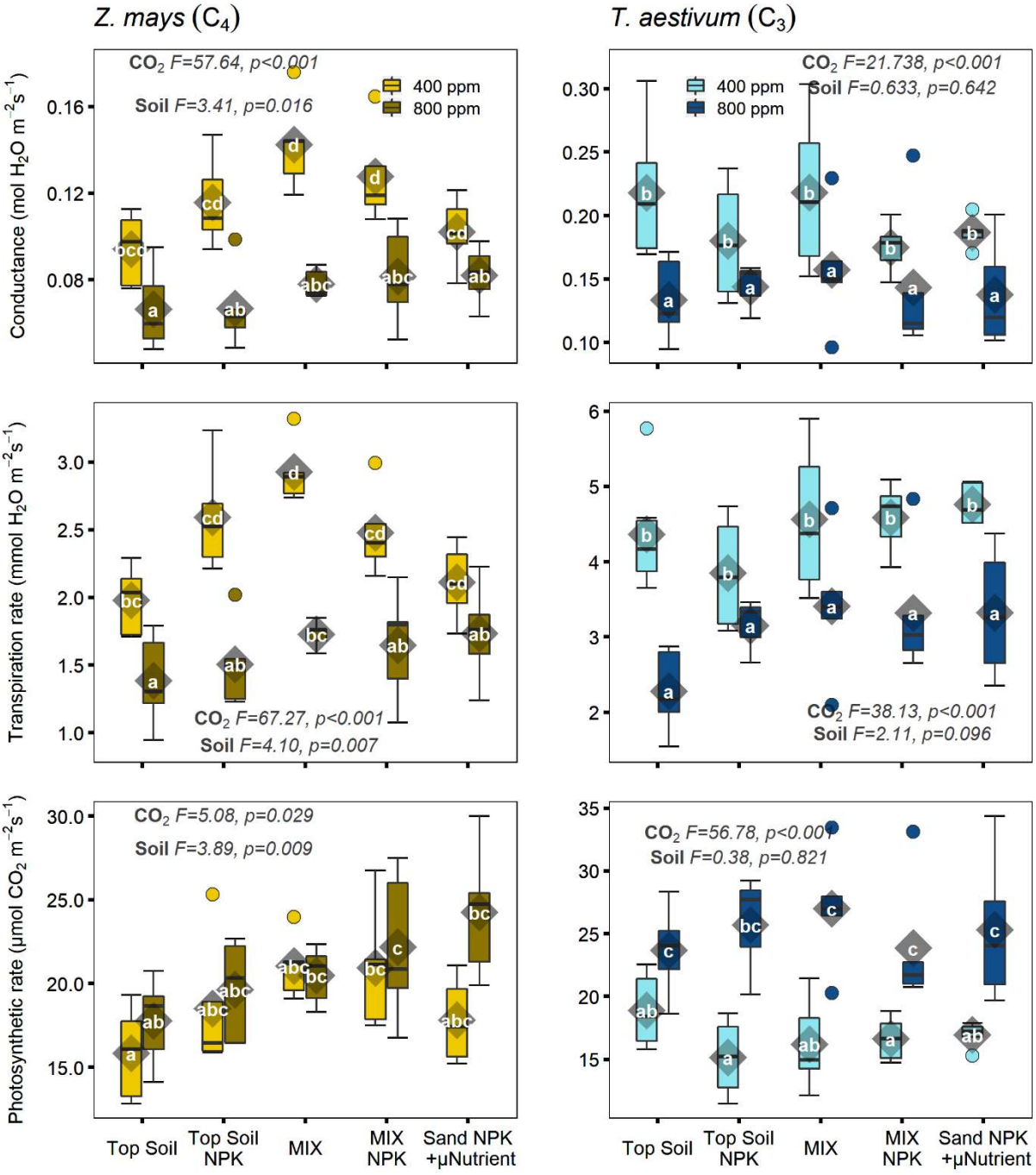
Figure 1



911

912 **Figure 2**

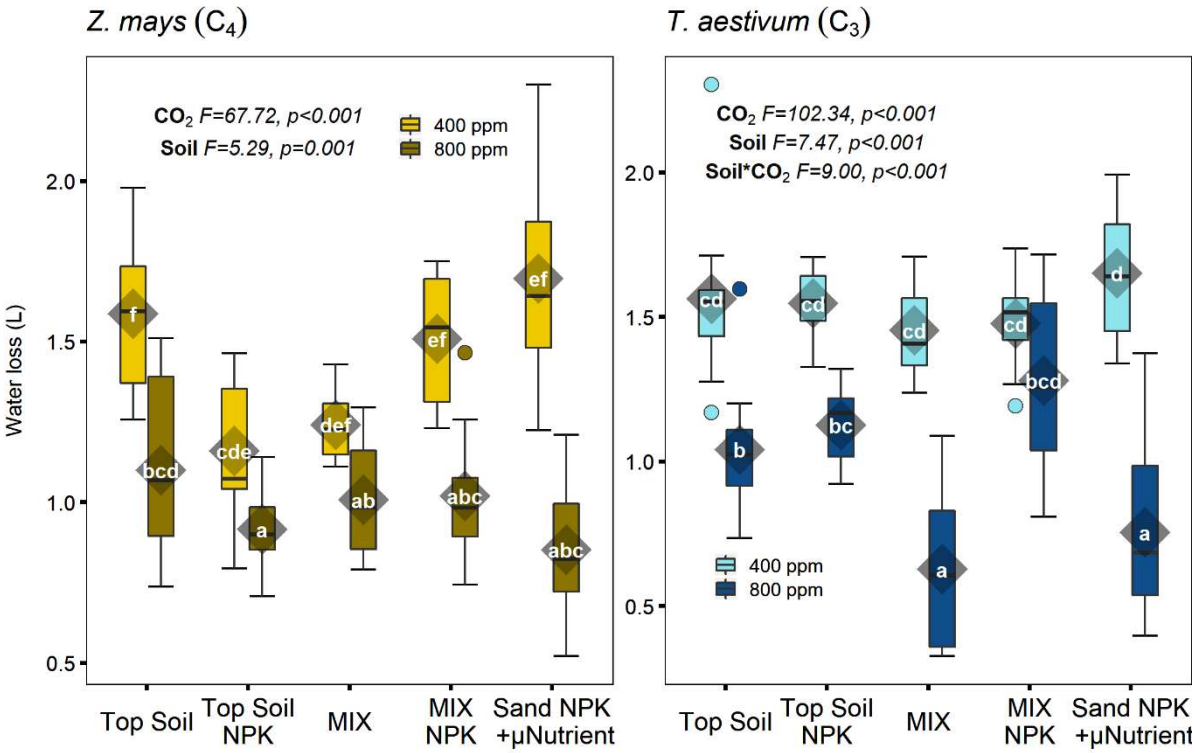
913



914

915 **Figure 3**

916



917

918 **Figure 4**

919

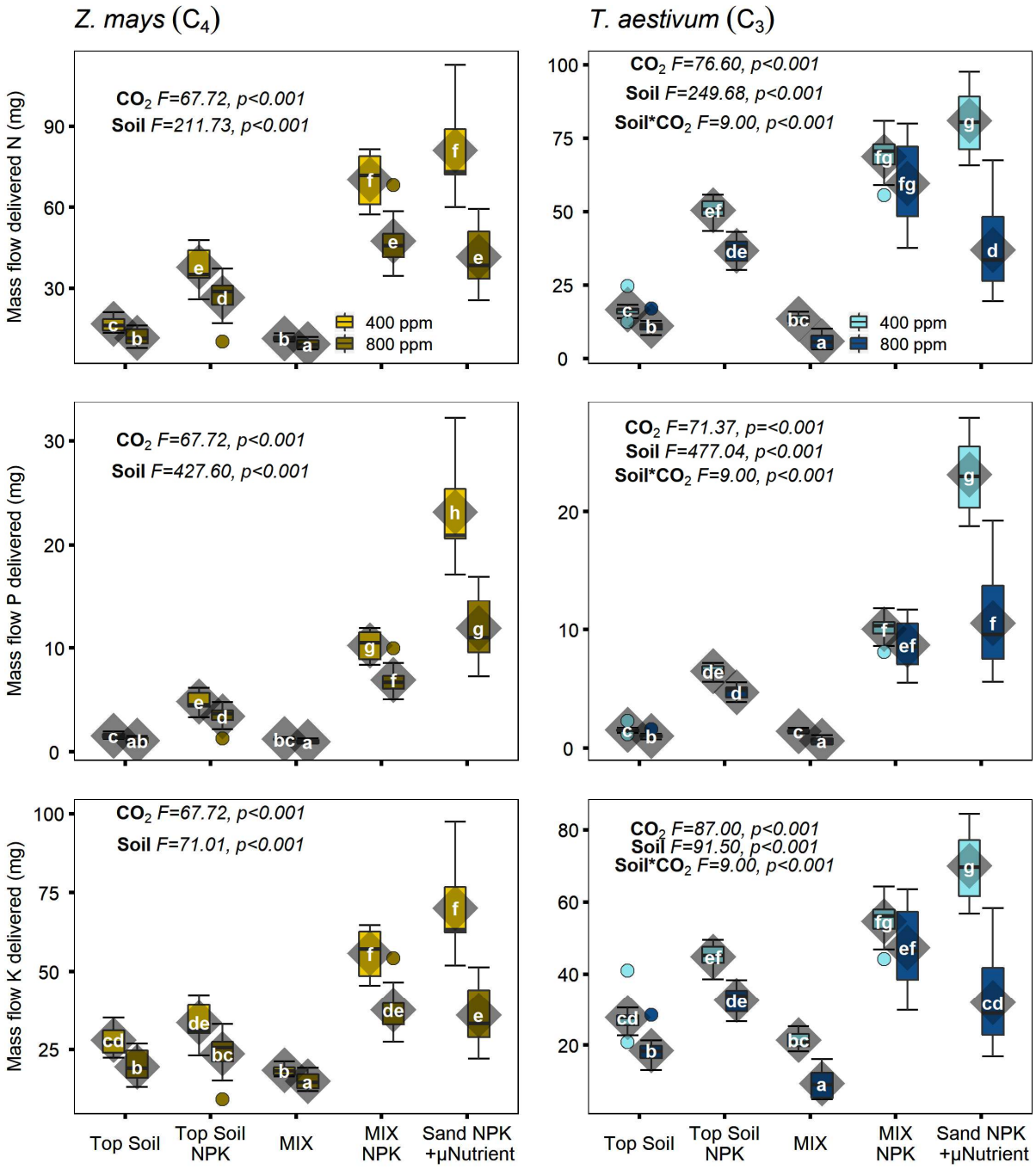

Z. mays (C4)
T. aestivum (C3)
Mass flow delivered N (mg)
Mass flow P delivered (mg)
Mass flow K delivered (mg)
CO2 F=67.72, p<0.001
Soil F=211.73, p<0.001
CO2 F=76.60, p<0.001
Soil F=249.68, p<0.001
Soil*CO2 F=9.00, p<0.001
CO2 F=67.72, p<0.001
Soil F=427.60, p<0.001
CO2 F=71.37, p=<0.001
Soil F=477.04, p<0.001
Soil*CO2 F=9.00, p<0.001
CO2 F=67.72, p<0.001
Soil F=71.01, p<0.001
CO2 F=87.00, p<0.001
Soil F=91.50, p<0.001
Soil*CO2 F=9.00, p<0.001
400 ppm
800 ppm
Top Soil
Top Soil NPK
MIX
MIX NPK
Sand NPK +µNutrient

Figure 5

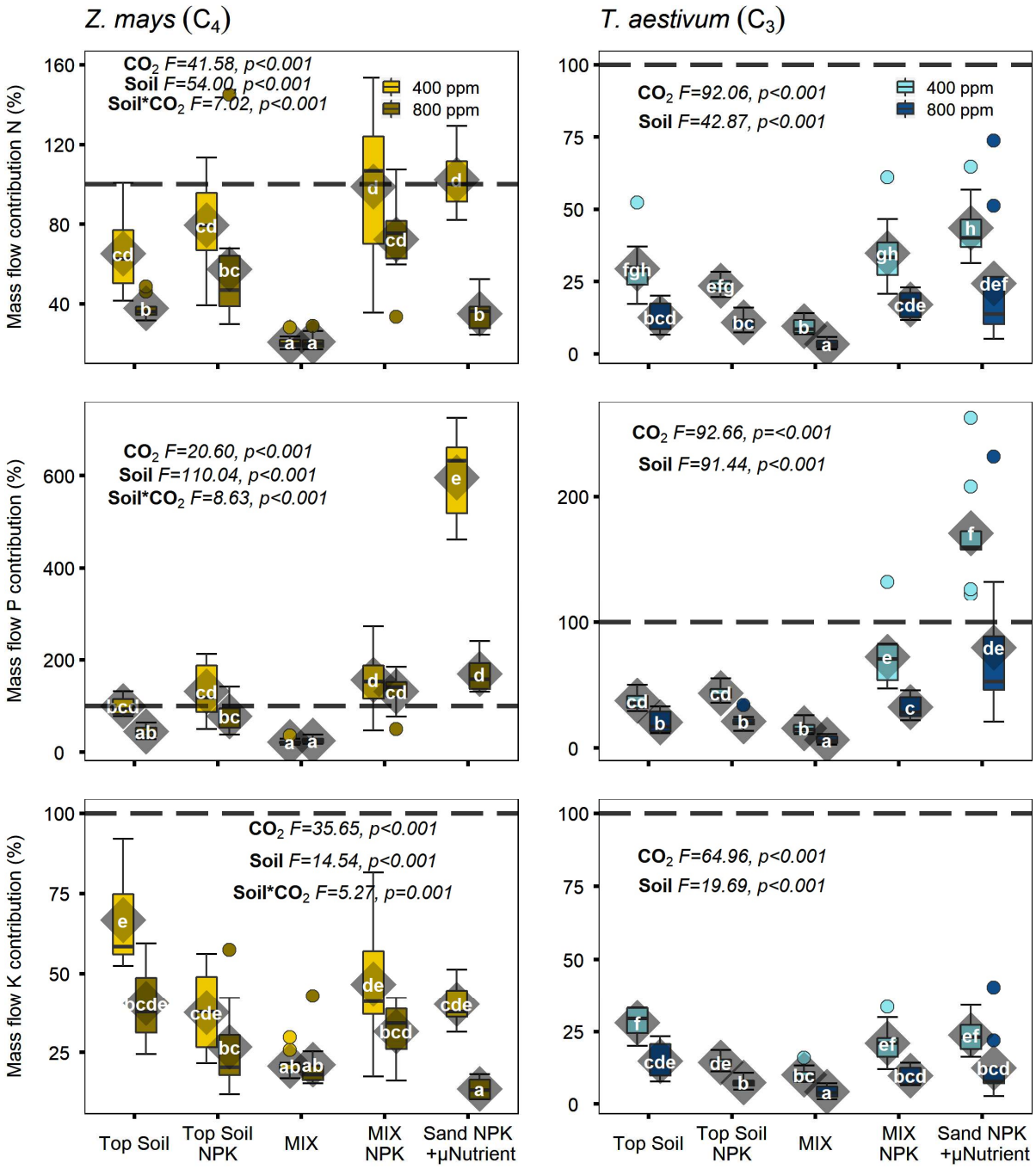
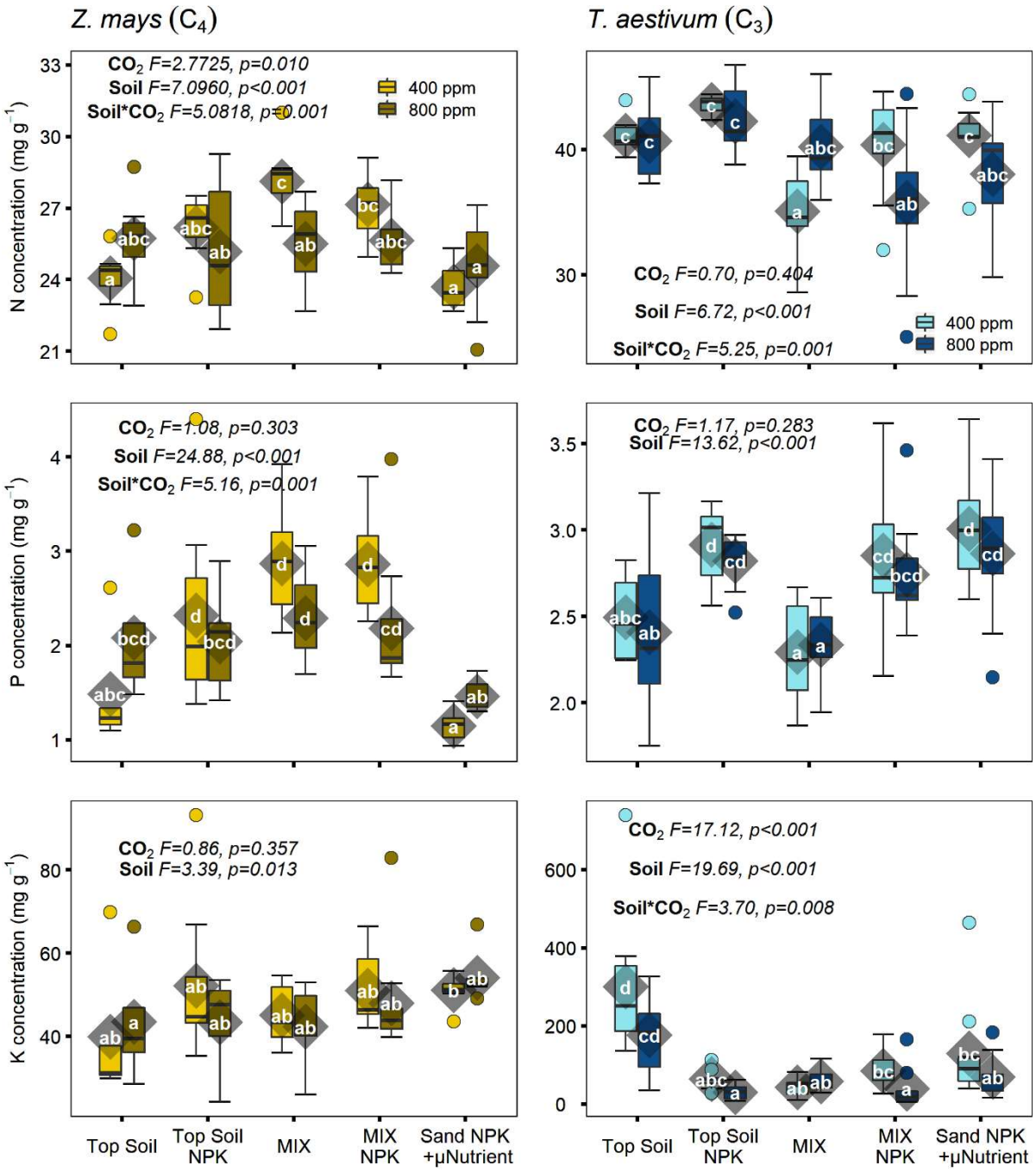

Z. mays (C4)
T. aestivum (C3)
Mass flow contribution N (%)
Mass flow P contribution (%)
Mass flow K contribution (%)
CO2 F=41.58, p<0.001
Soil F=54.00, p<0.001
Soil*CO2 F=7.02, p<0.001
CO2 F=92.06, p<0.001
Soil F=42.87, p<0.001
CO2 F=20.60, p<0.001
Soil F=110.04, p<0.001
Soil*CO2 F=8.63, p<0.001
CO2 F=92.66, p=<0.001
Soil F=91.44, p<0.001
CO2 F=35.65, p<0.001
Soil F=14.54, p<0.001
Soil*CO2 F=5.27, p=0.001
CO2 F=64.96, p<0.001
Soil F=19.69, p<0.001
400 ppm
800 ppm
Top Soil
Top Soil NPK
MIX
MIX NPK
Sand NPK +µNutrient

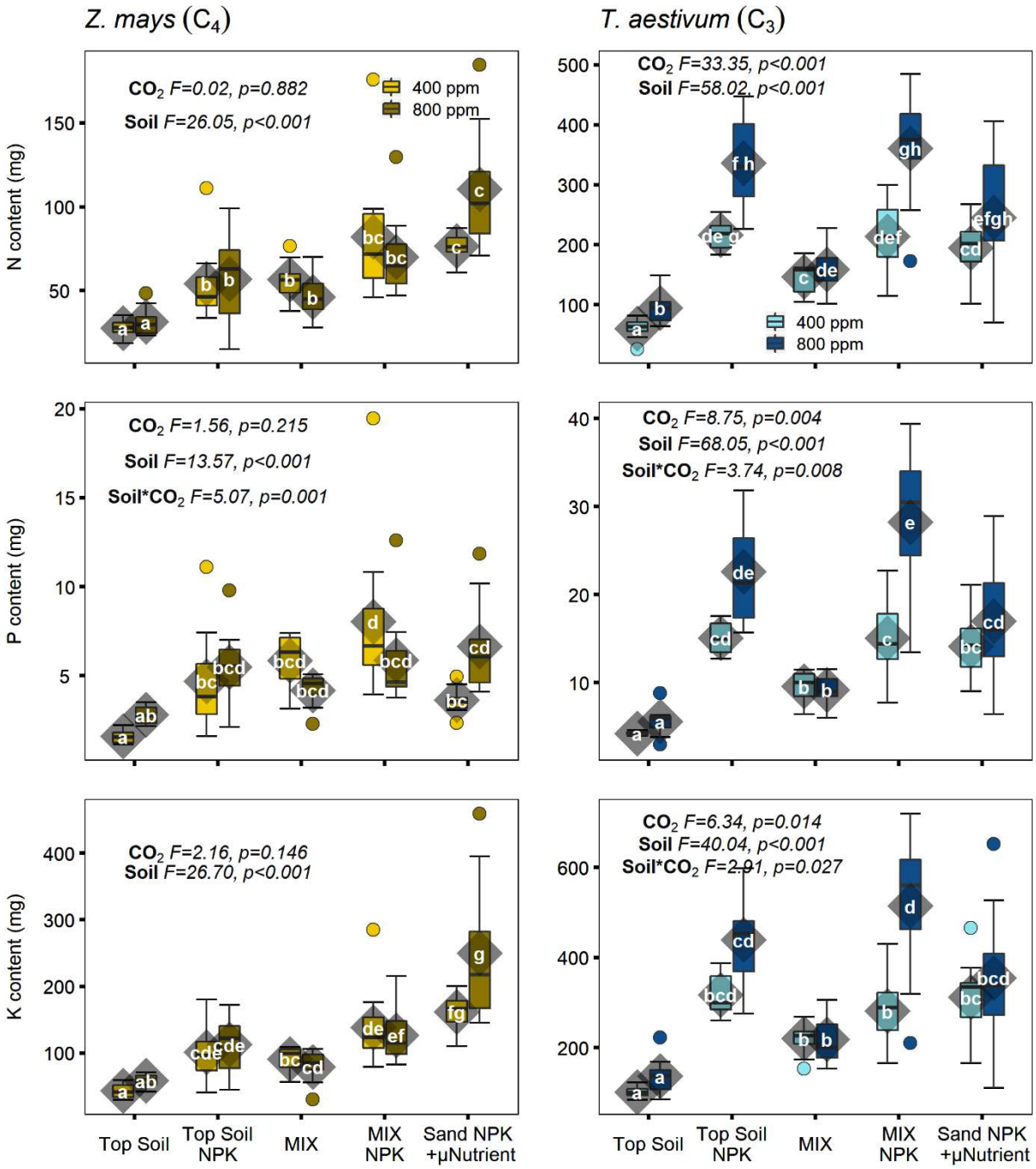
Figure 6



926

927 **Figure 7**

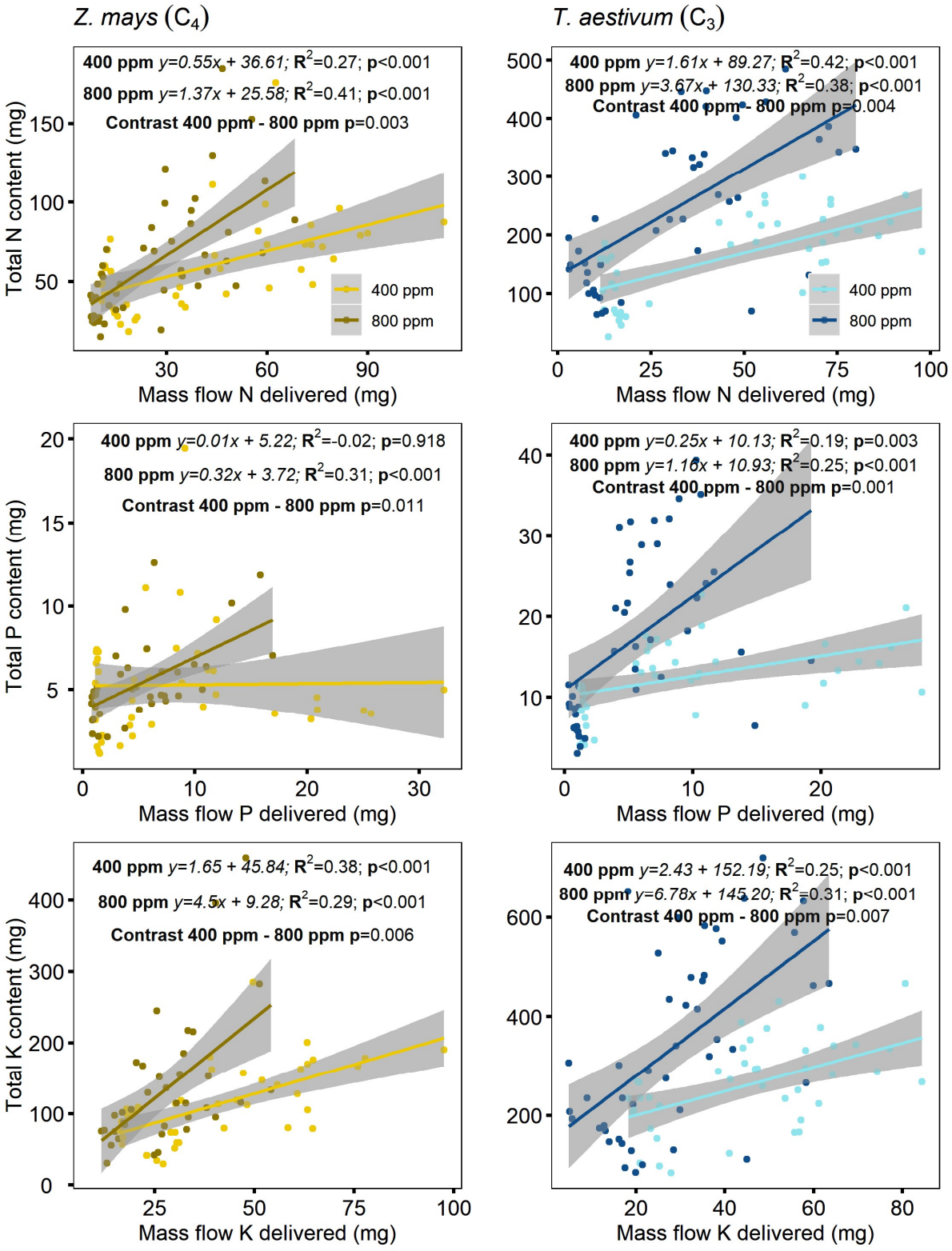
928



929

930 **Figure 8**

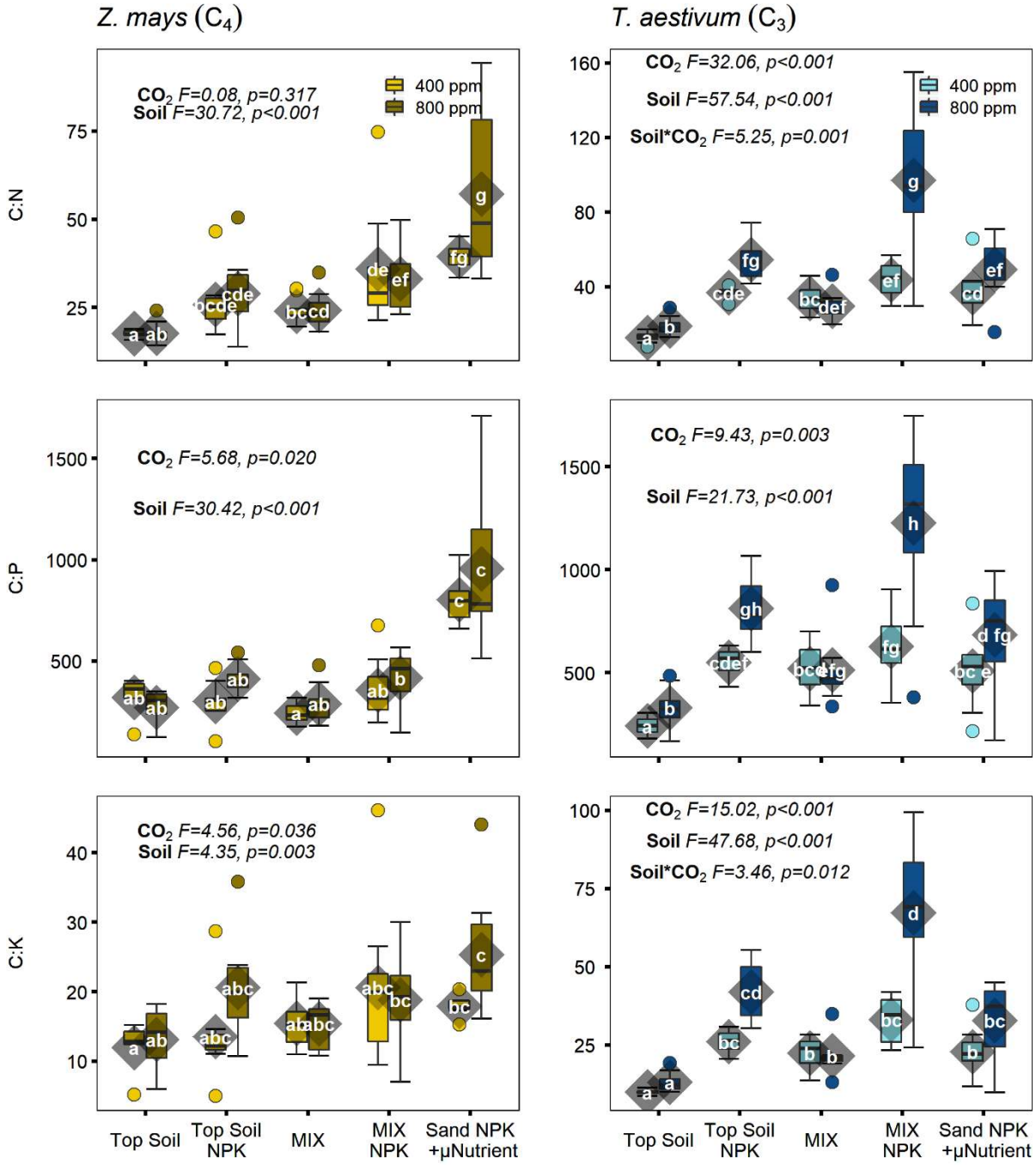
931



932

933 **Figure 9**

934



935

936 **Figure 10**

937

Chapter 3

The effects of low vapour pressure deficit on the nutrient status of maize and wheat under well-watered conditions

Abstract

The effects of varying VPD levels on mass flow, biomass accumulation and nutrient status were investigated in maize and wheat plants. Plants were grown at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa in well-watered Soil and Sand. To demonstrate the importance of mass flow, plants grown in Sand were supplied nutrients in such a way that they had to rely exclusively on mass flow or diffusive processes (i.e. not interception) for nutrient delivery to their rhizosphere. Relative to the 1.613 kPa VPD level, cumulative water use (CW_L) was decreased on average by 40% and 17% at the 0.773 kPa level in the maize and wheat plants respectively, and by 48% and 35% at the 0.350 kPa level. In both soil treatments, mass flow contribution of most nutrients was in excess of tissue content in both species, even though a significant decrease was measured with decreasing VPD. Photosynthetic rates and stomatal conductance were little affected by VPD, but plants always accumulated more biomass at 0.773 kPa. Reduced CW_L may have impeded root-to-shoot transport of ions and reduced dry biomass accumulation in the maize and wheat plants at 0.350 kPa (-40% and -22% on average respectively, compared to 0.773 kPa plants). Tissue [NPK] was also lower (-13% [N], -41% [P] and -47% [K] respectively) in the 0.350 kPa VPD Sand-grown wheat plants, while increases in the proportion of finer roots may have alleviated reduced CW_L effects on tissue [NPK] in the C_4 species. Growth and nutrient concentrations in the maize and wheat plants were affected by their respective physiological and morphological response to varying VPD levels, rather than by changes in mass flow. The findings from this study suggest that negative effects of reduced mass flow may be alleviated under well-watered conditions.

Introduction

The difference in vapour pressure deficit (VPD) between leaf tissues and the atmosphere drives water evaporation from plants (Lihavainen et al., 2016). Low VPD decreases evaporative demand from the atmosphere, and as such, decreases plant transpiration rates, while the opposite is observed when plants are exposed to high VPD conditions (Adams & Hand, 1993; Yang et al., 2012). Varying VPD can have important effects on the growth and nutritional status of plants. An increase in air humidity decreases evaporative demand from the atmosphere and promotes stomatal opening, thereby facilitating CO_2 influx into the leaf (Bunce, 1984). Therefore, low VPD levels tend to increase biomass accumulation in plants (Leuschner, 2002; Lihavainen et al., 2016). However, the uptake of nutrients by the root systems of plants growing at low VPD may be reduced due to decreases in mass flow delivery of nutrients as a result of decreased plant transpiration (Barber, Walker & Vasey, 1963; Cramer, Hawkins & Verboom, 2009). Similarly, in a meta-analysis done by Mcgrath & Lobell (2013), a decline in nutrient concentrations due to reductions in transpiration has been reported in a variety of crops grown at elevated atmospheric [CO_2] ('eCO_2').

Transpiration-driven mass flow has been shown to be an important process affecting below-ground nutrient uptake in plants (Barber, 1995; Cramer, Hoffmann & Verboom, 2008). For example, Barber (1995) estimated that mass flow contributed to 79% N, 5% P, 18% K, 73% Ca, 88% Mg and 95% S in *Zea mays* plants growing in a fertile loam. Apart from air humidity and atmospheric [CO_2], plant transpiration can also be regulated by availability of below ground nutrients. In a study done by Cramer, Hoffmann & Verboom (2008), a first set of *Ehrharta calycina* plants had direct access to nutrients via interception, mass flow and diffusion, while a second set had to rely exclusively on mass flow or diffusive processes for nutrient delivery and uptake. As a result, the plants in the second set had a 63% higher transpiration rate and a 72% higher stomatal conductance, thus showing a nutritional regulation

of mass flow in this C_3 plant species. In a similar experimental setup, Rose et al. (2018) showed that C_4 grasses were unable to regulate their water flux in response to varying nutrient availability and accessibility. Thus, C_4 plants can potentially be more severely affected than C_3 plants in terms of their nutrition under conditions of reduced transpiration and low nutrient availabilities.

Several mechanisms, in addition to increased stomatal opening (Bunce, 1984; Gisleröd & Nelson, 1989; Lihavainen et al., 2016), have been proposed for the reported increases in biomass accumulation in C_3 plants under humid conditions. Such mechanisms include increases in leaf turgor and leaf water potential (Leuschner, 2002), which has been shown to increase leaf expansion in plants (Acevedo, Hsiao & Henderson, 1971; Kramer & Boyer, 1995). Altered root morphology is another mechanism that has been reported to lead to increases in biomass in plants growing at elevated air humidity. In a long-term study comparing the respective responses of silver birch and aspen trees to varying VPD levels, Rosenvald et al. (2014) initially found a decrease in growth in both species when grown at low VPD. However, while suppression of growth persisted in the aspen trees, the silver birch trees acclimatised to the high humidity environment and increased biomass accumulation by increasing the proportion of fine-root biomass and altering root morphology. This suggests that the response to increasing air humidity is not necessarily uniform amongst C_3 species.

In contrast, low humidity tends to decrease biomass accumulation in C_3 plants. For example, reductions in yield have been reported in wheat, kale, sugar beet (Ford & Thorne, 2016) and soya beans (Woodward & Begg, 1976) when exposed to high VPD levels. Reduced growth at low humidity may be explained by leaf water stress arising as a result of the rate of water uptake by the root systems being insufficient to meet the water demand of high transpiration rates (Hoffman, 1979; Ranathunge, Steudle & Lafitte, 2003; Kuwagata et al., 2012). Additionally, decreases in stomatal conductance to prevent excessive water loss under

1012 conditions of high evaporative demand (Mott & Parkhurst, 1991; Oren et al., 1999; Sinclair,
1013 Zwieniecki & Holbrook, 2008) can lead to restriction of growth in C_3 plants. While
1014 transpiration and stomatal conductance tend to increase with increasing VPD, often these
1015 increases tend to reach a maximum at VPD levels above 2.0 kPa (Turner, Schulze & Gollan,
1016 1985; Comstock & Ehleringer, 1993), which has been demonstrated to reduce growth in wheat
1017 as a result of reduced CO_2 influx (Condon et al., 2002). The stomatal and transpiration response
1018 of C_3 crops to increasing VPD levels can however be modulated by the water status of the soil
1019 in which the plants are growing. For example, Xue et al. (2004) experimentally demonstrated
1020 that transpiration rates in winter-wheat increased with increasing VPD under well-watered
1021 conditions, but decreased with increasing VPD under water-stressed conditions. Thus, C_3
1022 plants exposed to high VPD levels, but with adequate water supply may show little to no
1023 decreases in their nutrient status as a result of unrestricted mass flow delivery of nutrients.

1024 Since the photosynthetic apparatus of C_4 plants is usually saturated at ambient CO_2 (Ziska &
1025 Bunce, 1997), it is argued that decreased stomatal conductance at high VPD levels will have
1026 little to no effect on net photosynthetic CO_2 assimilation in these plants (Bunce, 1983; 1993).
1027 In a study examining the effects of eCO_2 on stomatal sensitivity to varying VPD levels,
1028 assimilation rate was not altered by varying VPD levels neither at ambient nor at eCO_2 in
1029 *Amaranthus hypochondriacus*, a C_4 species, while in *Helianthus annuus*, a C_3 species,
1030 insensitivity of stomatal conductance to changes in VPD was only found at eCO_2 (Bunce
1031 1993). In another study, no net effect of low air humidity was found on photosynthesis and
1032 biomass accumulation in a C_4 plant, *Panicum maximum*, even though stomatal conductance
1033 was reduced (Kawamitsu, Yoda & Agata, 1993). In that same study, it was found that
1034 stomatal closure induced by low air humidity led to reductions in photosynthesis and carbon
1035 assimilation in *Oryza sativa*, a C_3 crop. Similarly, in the Negev desert of Israel, Lange et al.
1036 (1975) demonstrated that decreases in stomatal conductance led to greater decreases in net

photosynthesis at low VPD in C_3 plants, compared to C_4 plants, in which the reductions were minimal. This suggests that C_4 plants are potentially more resilient against reductions in growth than C_3 plants under low air humidity conditions. However, in a study comparing the effects of high VPD on photosynthesis and stomatal conductance in four C_4 species, Bunce (1982) showed that stomatal conductance and photosynthesis were decreased, in *Amaranthus hybridus, A. hypochondriacus* and *Zea mays*. However, in *Portulaca oleracea*, while stomatal conductance decreased, no decrease in net photosynthesis was observed. The author argued that increased mesophyll conductance, a nonstomatal limitation to photosynthesis, was responsible for the lack of change in photosynthesis in *P. oleracea* at high VPD. The above finding therefore suggests that the response to increasing VPD levels is not necessarily uniform amongst C_4 species. The photosynthetic response of C_4 species under humid conditions seem to however be similar to C_3 species. When comparing the effects of VPD on two C_4 species, *Amaranthus hydridus* and *Portulaca oleracea,* and a C_3 species *Chenopodium album*, Bunce (1983) found that net photosynthesis was increased in all three species when humidity levels were increased, which the author attributed to increased stomatal opening in all the species, leading to increased influx of CO_2. While there is evidence for the effects of varying VPD levels on the growth and development of C_4 plants (Bunce, 1983; Kawamitsu, Yoda & Agata, 1993; Yang et al., 2012), the effects of varying VPD levels on mass flow of nutrients has seldom been considered in these plants, even though changes in stomatal conductance and transpiration have been documented (Tanner & Beevers, 1990).

Increased biomass accumulation is often observed in plants growing at low VPD, since stomatal opening and increases in leaf expansion due to increases in leaf turgor pressure are favoured under humid conditions (Acevedo, Hsiao & Henderson, 1971; Kramer & Boyer, 1995; Leuschner, 2002; Lihavainen et al., 2016). However, decreases in transpiration at low VPD as a result of reduced evaporative demand can also negatively impact growth and nutrient

concentrations in plants. Tullus et al. (2012) measured a 30% decrease in xylem sap flow, a reduction in growth rate, as well as a significant decrease in leaf N content and concentrations in aspen trees growing in a high humidity environment. The authors attributed this decrease to an impairment of N uptake as a result of decreased mass flow. Additionally, Sellin et al. (2013) experimentally showed decreases in growth and in leaf concentrations of N and P in silver birch trees grown under elevated air humidity, also attributed to a decrease in water flux as a result of decreased transpiration rates. However, Tanner & Beevers (1990; 2001) experimentally demonstrated that a decrease in transpiration rates in C_3 and C_4 plants hydroponically grown under humid conditions did not affect growth or nutrient concentrations. Additionally, Leuschner (2002) did not find any correlations between transpiration rates and nutrient concentrations in eight herbaceous plants grown hydroponically at low VPD levels and attributed the decrease in nutrient concentrations to dilution effects as a result of increased growth. While Cramer, Hawkins & Verboom (2009) argue that transpiration has an important role in nutrient delivery to the rhizosphere of plants grown in soil, they argue it plays no role in terms of the uptake of minerals across the root membrane, which are instead achieved by transporters such as the Ammonium Transporter (AMT) or the Nitrate Transporter (NRT) for the uptake of NH_4^+ or NO_3^- respectively (Crawford & Glass, 1998; Gojon et al., 2011; Xu, Fan & Miller, 2012). This potentially explains why a decrease in transpiration at low VPD does not alter the tissue nutrient status of plants grown hydroponically. However, in their experiment, Tanner & Beevers (1990) included a treatment of maize plants grown in soil and again found no effect of elevated air humidity (and consequent reduction in transpiration) on the nutrient status of these plants. Contrasting findings from the literature in terms of the effects of reduced transpiration on the nutrient status of plants grown under humid conditions therefore calls for a better understanding of the role and importance of mass flow delivery of nutrients.

In this study, I tested the effects of varying VPD levels on water use, biomass accumulation and nutrient status in maize and wheat plants. Experiments in which plants are grown at low VPD levels allow isolation of the effects of decreased transpiration on nutrient concentrations and delivery in plants. Although eCO_2 also leads to decreased transpiration rates in both C_3 and C_4 plants (see Chapter 2), it also has effects on other physiological process (i.e. photorespiration, Rubisco investment, root architecture and physiology), whereas VPD levels mainly affect transpiration rates. Thus, I expect decreasing VPD to affect biomass accumulation and nutrient status in a similar manner in wheat (C_3) and in maize (C_4). The experiment was performed under well-watered conditions so as to identify VPD effects on plant physiology independently of soil-water effects. Plants were grown at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa in well-watered Soil and Sand. To demonstrate the importance of mass flow, plants grown in Sand were supplied nutrients in such a way that they had to rely exclusively on mass flow or diffusive processes (i.e. not interception) for nutrient delivery to their rhizosphere. I first hypothesised that plant tissue nutrient concentrations and contents will decrease in both species with decreasing VPD as a result of reduced transpiration-driven mass flow of nutrients, and because of dilution effects due to increased biomass accumulation. I then hypothesised that root morphology will be altered in both species as a result of decreased delivery of nutrients by mass flow with increasing air humidity. Finally, I hypothesised that reduced mass flow at lower VPD levels will have a stronger effect on the nutrient status of plants that had to rely exclusively on mass flow or diffusive processes for nutrient uptake belowground (i.e. plants grown in the Sand treatment).

Methods

Plant germination and growth conditions

Wheat (*Triticum aestivum* c. SST015, Kaap Agri, Malmesbury, 7300, South Africa) and maize (*Zea mays* c. CAP 122-60, Capstone Seeds, Howick, 3290, South Africa) plants were grown from seeds soaked in aerated water for 24 h and 48 h, respectively, before being sown in trays of vermiculite. The trays were kept moist until the emerging seedlings were established. The seedlings were transplanted into 0.20 m diameter pots, each respectively containing 2 kgs of soil. 2 different soils were used in this experiment: a soil with high clay content (hereafter referred to as 'Soil'), and a sand with no clay content (hereafter referred to as 'Sand'). The pots containing Sand were equipped with a cylindrical central compartment to which plant roots did not have access. These cylinders housed the fertiliser pellets so that plants growing in Sand had to rely exclusively on transpiration-driven mass flow and diffusion so as to acquire nutrients released from the pellets (Cramer, Hoffmann & Verboom, 2008). A hole was cut into the base of each pot into which the central compartment was inserted and filled with sand. These cylinders were made from PVC pipes (0.5 m diameter, 0.18 m long, 0.003 m wall thickness). Holes of 0.0045 m in diameter were drilled into the cylinders, from top to bottom.. The holes were covered with strips of 50 µm nylon mesh (Nylon 270, Ecotoa Enterprises, Kwadukuza, South Africa) on the outer surface. Maize and wheat growing in Sand received a Long-Ashton solution containing 4 mM $NaNO_3$, 0.67 mM NaH_2PO_4, 1.5 mM Na_2HPO_4, 2 mM K_2SO_4, 1.5 mM $MgSO_4.7H_2O$, 4 mM $CaCl_2.2H_2O$, 0.1388 mM H_3BO_3, 0.0208 mM $MnSO_4.4H_2O$, 0.0023 mM $ZnSO_4.7H_2O$, 0.0033 mM $CuSO_4.5H_2O$, 0.0002 mM $Na_2MoO_4.2HO$ and 0.0899 Fe EDTA (Hewitt, 1966) every third day during the first 9 days of growth. These plants were first supplied with excess water before being supplied with 200 mL of the Long-Ashton solution. After the establishment period, 25 g of Osmocote® Exact® Patterned Release Fertilizer (The Scotts Company Ltd, c/o Bord na Móna, Main street, Newbridge, County Kildare, Dublin, Ireland) pellets containing (in %) 15 N; 4 P; 7.5 K; 1.8 Mg; 0.02% B; 0.047 Cu; 0.40 Fe; 0.06 Mn; 0.020 Mo and 0.015 Zn were introduced into the central PVC compartment in such a way

so as to distributed them across the depth of the pot. Plants growing in Soil did not receive any fertiliser throughout the growth experiment as the amount of available nutrients in this soil was deemed adequate (see below) to sustain growth of maize and wheat plants throughout the experiment. These soil types and fertiliser treatments were replicated so as to have wheat and maize plants subject to them in high, intermediate and low VPD levels. The experiment was conducted in three growth rooms, set at either 55%, 75% or 95% RH. RH and temperature in the growth rooms were measured twice daily with a Brannan whirling hygrometer (1HYBR-137452, Prospectors Supplies Pty Ltd, Australia). The average RH and temperature during the growth experiment in the growth room set at 55% RH were 49.82% and 25.25 °C (corresponding VPD = 1.613 kPa), 76% and 25.28 °C (corresponding VPD = 0.773 kPa) in the 75% RH room, and 89.12% and 25.25 °C corresponding (VPD = 0.350 kPa) in the 95% RH room.

Field capacity and water use

Prior to running the experiment, the respective field capacities of the two soil types were determined. This was done by supplying excess water to pots containing 200 g of Soil or Sand that had been oven-dried 7 d at 70°C to remove any moisture. The pots were covered with plastic sheets to prevent evaporation and were allowed to settle for 24 h. Field capacity of the different soil types was then determined as the dry weight percentage of the difference between the dry weight of the soil and the wet weight after 48 h after they had settled (Kirkham, 2005). The field capacities were 26 and 12% for the Soil and the Sand respectively. Throughout the experiment, the soils were kept between 70-100% of their respective field capacities by gravimetric measurement of water content. These field capacities were chosen to allow unrestricted mass flow (Marschner & Rengel, 2011). Water use of plants and evaporation rates differed between the VPD treatments. Typically, in high VPD conditions, plants were watered daily, every two days in the intermediate conditions, and every 3 days in the low VPD

conditions. For each soil type, 7 additional control pots filled with the same amount of soil (2 kg) were randomly distributed in the three growth rooms set at either 55%, 75% or 95% RH. These control pots were subject to the same watering regimes as the pots with the plants. This was done so as to estimate the cumulative water use per plant throughout the growth experiment by gravimetry. Guttation was observed every morning at the tip of the leaves on most maize plants growing in Soil at the low VPD level during the last two weeks of the experiment.

Soil nutrient availability in Soil

A soil column was constructed from a 20-mL syringe with the plunger removed, and a disc of Whatman No. 1 filter paper was placed at the bottom. The filter paper was topped with glass wool to prevent loss of sediment. Oven-dried soil was filled to the 20 mL mark and water added to field capacity. The soil column was inserted through a hole in the lid of a 50-mL Falcon centrifuge tube, placed in the tube, and centrifuged (Eppendorf Centrifuge 5810R) at 150 g for 2 min. The volume of water collected in the centrifuge tube was taken as a relative measure of the mass flow displaceable soil solution. The mass flow technique using low-speed centrifugation was a slight modification of the gravity method described by (Barber, 1995a). The elemental determination in the water extracts were performed by the Elsenburg Laboratory (Western Cape Department of Agriculture, Stellenbosch, South Africa, 33.845259 S; 18.834722 E). The concentrations of P, K, Ca, Cu, Fe, Mn and Zn were determined directly using a Radial ICP Spectrometer ICAP 7600 (Thermo Fisher Scientific, Sunnyvale, CA, USA), while N was determined on a BR-71394 DA Gallery Analyzer (Thermo Fisher Scientific, Sunnyvale, CA, USA). The water extracts from the Soil contained (in mg l^{-1}) 112.3 N, 23.4 P, 290 K, 291 Ca, 0.038 Cu, 0.01 Mn, 0.3 Fe and 0.126 Zn.

Gas exchange measurements

All plant gas exchange measurements were conducted on fully expanded leaves using a LI-6400 (Li-Cor, Lincoln, NE, USA) Portable Photosynthesis System equipped with 2 x 3 mm broadleaf cuvette and an integrated red/blue LI-6400-02B LED light source (Li-Cor, Lincoln, NE, USA), on well-watered plants. I set the RH in the cuvette to either 55%, 75% or 95% depending on the RH level to which the plants being measured were exposed. Reference CO_2 was set to 400 ppm and block temperature was set to 25°C, to match the temperature at which the plants were growing at. The variables measured were photosynthetic rate, stomatal conductance, internal leaf carbon and transpiration rate. Water use efficiency (WUE) was then calculated as the ratio of photosynthetic rate to transpiration rate.

Plant harvest

The wheat and maize plants were harvested after 4 weeks of growth. The soil was carefully washed away with water on a 2 mm sieve to minimise root loss. The plants were separated into root and shoot, dried with paper towel, and immediately weighed to obtain fresh weight. The leaves were then scanned with an LI-3100 Area Meter (Li-Cor, Lincoln, NE, USA) so as to obtain the leaf area and the specific leaf area (SLA) of each plant. The harvested plants were then oven-dried at 70°C for 48 h, after which dry weight was determined. The difference between foliar fresh weight and foliar dry weight was divided by the foliar fresh weight to obtain foliar water concentration. A representative sub-sample that constituted at least 15% of the total fresh weight, and that included the whole length of the root system of each plant was carefully cut off (Vandamme et al., 2013) and used to determine average root diameter (mm) and total root surface area (m^2) using a STD4800 scanner and WinRHIZO version 2013a (Regent Instruments, Canada). Specific root length (SRL, m g^{-1}) was calculated as the total root length (m) divided by the root dry weight (g). The root characteristics were converted to whole-root results by multiplying by their respective conversion factors to 100% of the root.

Mass flow delivery and contribution

Mass flow delivery of nutrients was calculated as the cumulative amount of water transpired by a plant throughout the growth experiment multiplied by the concentration of that nutrient in the soil solution. Since delivery of a nutrient does not necessarily equate to acquisition, mass flow contribution to tissue concentration of that nutrient was then calculated as a percentage of the total concentration of that nutrient in the plant. The remaining nutrient content of the plants was assumed to have been acquired by diffusion and/or root interception (Barber, 1995).

Foliar elemental analyses

The dried foliar and root samples were ground to a fine powder using a grinder or a ball-mill (MM200, Retsch, Germany). The powdered samples were placed in Perspex rings sealed with 4 μm Polypropylene thin film (Chemplex Industries Inc, Florida, USA) and introduced to a SPECTRO XEPOS XRF spectrometer (SPECTRO, AMATEK materials analysis division, Kleve, Germany). Analyses were conducted using the X-LabPro 5 software, which incorporates the universal 'Turbo Quant Powders' method. The instrument was calibrated by using a certified standard GBW07312 (National Research Center for CRMs, Beijing, China), for which elemental concentrations were obtained from NOAA Technical memorandum NOS ORCA 68 (1992). Only the elements that were within the machine's detection limits were included.

The carbon isotope ratio ($\delta^{13}C$) and the total N concentration of the powdered foliar and root samples were determined using mass spectrometry. Samples were weighed into tin capsules (5 x 9 mm; Säntis Analytical, Teufen, Switzerland, with *ca*. 2 mg of foliar powder used for anaylsis). The samples were combusted in a Flash 2000 organic elemental analyser and the gases passed into a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (all from Thermo Scientific, Bremen, Germany). In-house standards and

one IAEA (International Atomic Energy Agency) standard (USGS25) were used to calibrate the results. Nitrogen concentration was expressed relative to atmospheric nitrogen (Evans, 2001).

Data analyses

All statistical analyses were performed using R Statistical Software (R Core Team, 2020). Comparisons of all the response variables were made using linear models with VPD level and soil type as explanatory variables, fitted as interactions, or as separate main effects if the interaction was not significant. Mass flow data was available only for plants growing in Soil, and thus comparisons were made using linear models with VPD levels as the explanatory variable, and mass flow delivery and mass flow contribution of nutrients respectively as response variables. Analyses of variance were done on all the fitted linear models to determine the significance of the main effects using the *'car'* package (Fox & Weisberg, 2011). Where main effects significantly explained variation, pairwise comparisons (Tukey post-hoc tests) of group levels means were then performed using the package *'emmeans'* (Lenth, 2018). All plots were generated using the *'ggplot2'* package (Wickham, 2016).

Figures 1 to 8 (in Chapter 3) and Appendices 13 to 21 show the effects of soil treatment and VPD on morphological, physiological and nutrient variables in the maize and wheat plants. The ANOVA results indicating the significance of each factor are shown in each plot, with interaction terms excluded if they were not significant ($p > 0.05$). Note that a significant interaction term implies that the effect of VPD varied between soil treatments.

Results

Total biomass, root biomass, shoot-to-root ratio and root characteristics

Total biomass was higher at the intermediate VPD level in both maize and wheat plants, in both soil treatments (Fig. 1). Root biomass was higher at the intermediate VPD level in maize plants in both soil types, while there was no difference in root biomass between the VPD levels within each soil type in wheat plants (Fig. 1). Shoot-to-root ratios were highest in maize plants growing at the intermediate VPD level in Sand, with no difference measured between the VPD levels in Soil (Fig. 1). There was no difference in shoot-to-root ratio between the VPD levels within each soil type in wheat plants.

Average root diameter and total root surface area were higher in maize plants growing at the intermediate VPD level in both Sand and Soil, while no difference in these variables was measured between the VPD levels within each soil type in wheat plants (Fig. 2). There was no difference in specific root length between the VPD levels within each soil type in maize plants (Fig. 2). In wheat, specific root length was lowest at the intermediate VPD level in Sand, and highest at the high VPD level in Soil.

Gas exchange measurements, water use and carbon isotopic ratios

Neither VPD nor soil treatment affected stomatal conductance or photosynthetic rate in maize (Fig. 3). Photosynthetic rate was not affected by VPD in wheat plants either. In wheat plants, stomatal conductance was lowest at the intermediate VPD level in both soil types. Transpiration rate was highest at the high VPD level and lowest at the low VPD level in maize in both soil types (Fig. 3). Transpiration rate in wheat plants was highest at the high VPD level in both soil types, with no difference observed between the two other VPD levels. Cumulative water use per plant measured gravimetrically was highest at the high VPD level and lowest at the low VPD level in maize and wheat plants in both soil types (Fig. 4). Variation in foliar water concentrations could potentially indicate if any water stress arose as a result of high transpiration rates. Water concentration was lowest in maize growing at the intermediate VPD

level in Soil, and lowest at the high VPD level in Sand (Fig. 4). There were no differences in foliar water concentrations amongst the VPD levels in wheat plants. WUE was highest at the low VPD level and lowest at the high VPD level in maize in both soil types (Fig. 5). In wheat plants, WUE was higher at the low VPD level in both soil types, with no difference measured between the two other VPD levels. In maize, $\delta^{13}C$ was more negative at the high VPD level and less negative at the low VPD level in both Sand and Soil (Fig. 5). In wheat plants in Sand, $\delta^{13}C$ was less negative at the high VPD level and more negative at the low VPD level, while in Soil it was marginally more positive at the low VPD level. Variations in $\delta^{13}C$ at the different VPD levels can inform about potential stomatal limitations to CO_2 assimilation in both species (see Discussion).

Mass flow delivery of nutrients and contribution to tissue content in Soil

Mass flow delivered N, P, K, Ca, Cu, Mn, Fe and Zn was highest at the high VPD level and lowest at the low VPD level in both maize and wheat plants growing in Soil (Fig. 6, Appendices 13 and 14). Contribution of mass flow to tissue content of N, P, K, Ca, Cu Mn, Fe and Zn in maize was the highest at the high VPD level, with no difference between the other VPD levels (Fig. 7, Appendices 15 and 16). In wheat plants, mass flow contributed N was highest at the high VPD level, while mass flow contributed Mn and Fe were lowest at the same VPD level. There was no difference in mass flow contributed P, K, Ca, Cu and Zn between the VPD levels in wheat plants. Contribution of mass flow to tissue content of N, P, K and Ca was above a 100% of tissue content at all VPD levels in maize and wheat plants growing in Soil (Fig. 6, Appendix 15). Additionally, mass flow contributed Cu, Mn, Fe and Zn were above 100% at all VPD levels in wheat plants growing in Soil (Appendices 15 and 16).

Tissue nutrient concentrations and contents

Tissue N, P, K concentrations were decreased at the high VPD level in both maize and wheat plants growing in Sand (Fig. 8). The concentrations of these nutrients were also decreased at the low VPD level in Sand grown wheat plants, but not in maize plants. Ca and Zn concentrations were the lowest Sand maize plants growing at the intermediate VPD levels, with little to no difference measured in Cu, Mn and Fe concentrations (Appendices 17 & 18). Little to no variation was measured in concentrations of Ca, Cu, Mn, Fe and Zn concentrations in Sand wheat plants. Concentrations of all the nutrients were lowest in Soil maize plants growing at the intermediate VPD level. In Soil wheat plants, concentrations of N, P, K and Ca did not vary between the VPD levels, while Cu, Mn and Fe concentrations were the highest at the high VPD level, and little to no difference was measured in Zn concentrations in these plants. The variation in the tissue content of all the nutrients in both maize and wheat plants, in both Sand and Soil, followed very similar patterns to total biomass at the different VPD levels (Appendices 19-21).

Discussion

As expected, transpiration rates and cumulative water use decreased with decreasing VPD in both maize and wheat, in both soil treatments. These decreases were mainly due to decreased evaporative demand, since stomatal conductance remained relatively unaffected by VPD in maize and was lowest at the intermediate VPD level in wheat. Additionally, VPD did not affect photosynthetic rates in either species. WUE increased with decreasing VPD, as a result of decreased transpiration rates. Since changes were nonetheless measured in biomass accumulation and nutrient concentrations in both species at the different VPD levels, the lack of variation in photosynthetic rate and stomatal conductance suggests that other factors such as

mass flow, soil water status, soil nutrient availability and accessibility, or dilution effects, influenced the growth and development of the plants at the different VPD levels.

Mass flow data was only available for the plants growing in the Soil. However, mass flow delivery of nutrients was likely decreased in a similar manner in Sand plants, since mass flow is calculated directly from cumulative water use, and cumulative water use was decreased in a similar manner in both soil treatments. Thus, reductions in mass flow, in both Soil and Sand, could potentially explain the reductions in biomass accumulation in the maize and wheat plants grown at the lowest VPD level. For example, in aspen and silver birch trees grown at low VPD, it has experimentally been demonstrated that decreases in mass flow, as a result of decreased transpiration, led to reductions in growth and foliar N content (Tullus et al., 2012; Sellin et al., 2015). In the present study however, mass flow contribution of N, P, K and Ca (as well Cu, Mn, Zn and Fe in wheat plants) was in excess of tissue contents of the maize and wheat plants, even at the lowest VPD level. The well-watered conditions (between 70-100% field capacity) to which the maize and wheat plants were subject at all the VPD levels and in both soil treatments may have allowed for excess delivery of these nutrients to the rhizosphere by mass flow, due to constant water flow, even though cumulative water use was reduced in the low VPD plants. Therefore, reductions in mass flow cannot fully explain the reductions in growth in the plants grown at this VPD level. One explanation could be that the low transpiration rates measured in both species at the low VPD level led to a reduction in xylem water movement, thus impeding root-to-shoot transport of ions within the plants. While Tanner & Beevers (1990; 2001) suggest that the quantity of transpiration-driven water flux in the xylem of plants considerably exceeds that required for movement of long-distance transport of ions, they do concede that this movement is usually accelerated by higher transpiration rates. For instance, transpiration mostly affects translocation rather than uptake of nutrients by roots in plants, but increased transpiration can nonetheless lead to increased ion uptake by increasing

1349 the flux of elements through the roots' apoplastic pathway, potentially favouring greater uptake
1350 into the symplastic pathway (Marschner & Marschner, 2012). While mass flow delivery of
1351 nutrient to the rhizosphere does not equate to nutrient acquisition, the reduced transpiration
1352 rates may have decreased the nutrient uptake potential of the roots in the maize and wheat
1353 plants at the low VPD level, subsequently leading to reductions in biomass.

1354 Tissue nutrient concentrations were, however, affected differently in the maize and
1355 wheat plants at the low VPD level. This contrast could be due to the decrease in root diameter
1356 at the low VPD level in the C_4 species, while no change in root diameter was measured in wheat
1357 plants (neither in Sand, nor in Soil) between the VPD levels. Increases in the proportion of fine
1358 roots have been demonstrated to be a response to low N or P availability (Gillespie, 1989; De
1359 Groot et al., 2003; Chapman et al., 2012; Luo et al., 2013) under conditions of varying VPD
1360 (Parts et al., 2013; Rosenvald et al., 2014). However, the ability for a plant to respond in terms
1361 of root morphology under conditions of low N or P availability and changing air humidity can
1362 differ between species, as well as between genotypes (Chapagain, 2014; Rosenvald et al.,
1363 2014). Thus, in the present study, the decrease in root diameter in the maize plants growing in
1364 Sand at the low VPD level may have allowed them to maintain adequate tissue concentrations
1365 of P and K compared to their wheat counterparts, even though transpiration rates and
1366 cumulative water use were decreased to a similar extent in both species. It has indeed been
1367 demonstrated that a morphological adaptation to low shoot P and low-P stress was an increase
1368 in the proportion of fine-root biomass in species such as soybean, sunflower or maize
1369 (Fernandez & Rubio, 2015; Wen et al., 2017). Furthermore, tissue nutrients concentrations in
1370 the wheat plants seem to have been affected differently depending on whether they grew in the
1371 Soil or in Sand. It is possible that reductions in transpiration combined with the restriction on
1372 root interception for nutrient uptake in the Sand treatment led to the lower P and K
1373 concentrations in the Sand wheat plants compared to the Soil wheat plants at the lowest VPD

level. Thus, there is good evidence to support the hypothesis that effects of reduced water flux with decreased VPD are exacerbated in Sand, where plants had to rely exclusively on mass and flow and diffusion.

In Soil maize plants, even though mass flow and biomass accumulation were reduced at the lowest VPD level, they maintained relatively high tissue concentrations of N, P, K, Ca and Zn compared to the ones growing at the intermediate VPD level. It is possible that guttation (in addition to increases in the proportion of finer roots), which was frequently observed on these plants during the growth experiment, may have facilitated the uptake and movement of nutrients within these plants even when transpiration rates were reduced (Tanner & Beevers, 1990; Slewinski, Meeley & Braun, 2009; Singh, 2016). Tanner & Beevers (1990) also observed guttation in maize plants growing under conditions where the air humidity was above 95%. This process has been shown to happen frequently in this C_4 crop (Erhardt, 1978) and potentially demonstrates its importance with regards to nutrient uptake and transport from the root to the shoot in plants.

In both soil treatments, maize and wheat plants growing at the high VPD level also experienced a reduction in biomass accumulation. This result is in agreement with previous studies where growth at low relative humidity was found to be reduced in various crop species (Woodward & Begg, 1976; Ranathunge, Steudle & Lafitte, 2003; Kuwagata et al., 2012; Ford & Thorne, 2016). The reduction of growth at low humidity in these studies was attributed to leaf water stress arising as a result of the rate of water uptake by the root systems being insufficient to meet the high transpiration rates. Significantly lower foliar water concentrations in the maize plants growing in Sand at the highest VPD level could potentially indicate that these plants were water-stressed as a result of high transpiration rates, thus leading to lower biomass. However, the maize plants growing in Soil, and the wheat plants in both soil treatments at the highest VPD level, did not have significantly lower foliar water

concentrations, suggesting that they were not water stressed. There were little to no changes in the shoot-to-root ratios between the VPD levels in both the C_3 and C_4 plant species, potentially suggesting that adequate root systems may have allowed the high VPD plants to avoid getting water-stressed, in addition to the well-watered status of the substrates. Thus, leaf water stress due to high transpiration rates cannot explain the reduction in growth in most of the plants grown at low air humidity in the present study. Furthermore, transpiration-driven mass flow of nutrients was highest in the high VPD plants, due to their high transpiration rates and cumulative water use. Why then is a reduction in biomass accumulation still observed in most of the plants grown at high VPD?

I suggest that stomatal and non-stomatal limitations to carbon assimilation may have led to the reductions in biomass observed in both species at the high VPD level. Gas exchange measurements did not reveal any differences in photosynthetic rates or stomatal conductance potentially because these measurements were always conducted after the plants had been watered, and thus any VPD-induced fluctuations in these two variables may have been masked. $\delta^{13}C$, on the other hand, is an integrated measure over the whole growth period of the plants and may be more indicative of any limitations to carbon assimilation throughout their development. The relationship between WUE and $\delta^{13}C$ is well-established in the literature (Farquhar et al., 1989; Condon, Richards & Farquhar, 1993; Wright, Rao & Farquhar, 1994; Brück, Payne & Sattelmacher, 2000). The opposite trends in $\delta^{13}C$ observed with increasing VPD (thus, with decreasing WUE) respectively in maize and wheat, are in agreement with the study done by Madhavan, Treichel & O'Leary (1991), with the differing discrimination of ^{13}C by Rubisco and PEP-Carboxylase in the C_3 and C_4 plants respectively explaining these trends (Farquhar, O'Leary & Berry, 1982; Winter et al., 1982). The respective $\delta^{13}C$ trends in the C_3 and the C_4 species potentially indicate that stomatal opening was favoured at low VPD, while closure was favoured at high VPD (Morison & Gifford, 1983; Bowman et al., 1989). Thus,

even though the gas exchange measurements did not detect differences in stomatal conductance, partial stomatal closure at the high VPD level (inferred from the $\delta^{13}C$ trends) may have restricted the influx of CO_2, potentially restricting carbon assimilation (Cornic, 2000; Chaves et al., 2002; Cernusak et al., 2019), thereby leading to decreases in biomass. Higher evaporative demand at high VPD nonetheless still leads to higher transpiration rate for a given stomatal aperture (Sanchez-Diaz et al., 2002), and may explain the highest measured transpiration rates and cumulative water use of the high VPD plants, even if there was partial stomatal closure. Apart from stomatal effects, Sharkey (1984) has experimentally demonstrated that high transpiration rates could lead to non-stomatal limitations to carbon assimilation as a result of reduced mesophyll capacity for photosynthesis, attributed to water potential drops within the mesophyll. Consequently, decreases in biomass accumulation due to stomatal and non-stomatal limitations in the maize and wheat plants grown at high VPD may explain lower concentrations of N, P and K in their tissue, as a result of lower demand for growth.

The higher biomass accumulation in both maize and wheat plants at the intermediate VPD level is likely due to the absence of the different limitations on growth imposed by the more extreme VPD level. However, in Soil-grown maize at the intermediate VPD level, a possible dilution effect of most of the nutrients is observed, as shown by their lowest concentrations. Similarly, Leuschner (2002) experimentally demonstrated that shoot nutrient concentrations in eight plants grown at low VPD levels decreased due to dilution effects as a result of carbon assimilation in plants grown at low VPD levels. In the present study, transpiration rate and cumulative water use of the maize and wheat plants grown at the intermediate VPD level were nonetheless significantly decreased relative to the plants growing at the highest VPD level. Thus, increased biomass accumulation, coupled with a reduction in transpiration, may have led to inadequate supply and movement of nutrients by mass flow, thereby leading to a dilution effect. This is especially true for nutrients such as Cu, Mn, Fe and

Zn which were not supplied in excess of physiological requirements by mass flow in the intermediate VPD maize plants. The same explanation may also be applicable to describe the dilution of Ca and Zn in the intermediate VPD maize plants growing in Sand, since mass flow was the main mechanism for the delivery of these nutrients to the rhizosphere in this soil treatment. The above findings demonstrate the potentially important role of mass flow for nutrient delivery and on plant nutrient status.

Conclusion

The findings from this experiment corroborate those from the eCO_2 experiment (Chapter 2), in that well-watered conditions allowed for the delivery of most nutrients by mass flow in excess of physiological requirements, in both species, even when transpiration rates and cumulative water use were reduced at the intermediate and lowest VPD level. As such, decreases in mass flow had little to no effect on nutrient concentration or biomass accumulation in either species. Rather, the physiological and respective morphological responses to VPD of the maize and wheat plants led to changes in biomass accumulation and nutrient concentrations at the different VPD levels.

List of figures

Figure 1: Variation in total biomass, root biomass and shoot-to-root ratio in maize and wheat plants, grown in Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. The boxes and horizontal lines represent the first and the third quartiles and the medians, respectively. The whiskers represent 1.5 × the interquartile range and outliers above/below are shown as points. The diamonds represent the mean values and the filled circles indicate

outliers. The F- and p-values of the main effects and interaction (when significant/present) from the analysis of variance done on the linear models fitted are displayed. Lower case letters indicate the significant differences between treatments as determined by Tukey's post-hoc comparisons.

Figure 2: Variation in average root diameter, total surface area and specific root length in maize and wheat plants, grown in Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics as in Figure 1.

Figure 3: Variation in stomatal conductance, transpiration rate, and photosynthetic rate in maize and wheat plants, grown in Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics as in Figure 1.

Figure 4: Variation in cumulative water use and foliar water concentration in maize and wheat plants, grown in two Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics as in Figure 1.

Figure 5: Variation in water-use efficiency and in $\delta^{13}C$ maize and wheat plants, grown in Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics as in Figure 1.

Figure 6: Variation in transpiration-driven mass flow delivery of N, P and K in maize and wheat plants, grown in Soil, at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics as in Figure 1. Corresponding air relative humidity (in %) at 25°C shown on the x-axis.

Figure 7: Variation in transpiration-driven mass flow contribution of N, P and K to tissue content in maize and wheat plants, grown in Soil, at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. The dashed line represents 100% contribution to tissue content. Statistics as in Figure 1. Corresponding air relative humidity (in %) at 25°C shown on the x-axis.

1494 **Figure 8:** Variation in total tissue concentrations of N, P and K in maize and wheat plants,
1495 grown in Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics
1496 as in Figure 1.

1497

1498

Figures

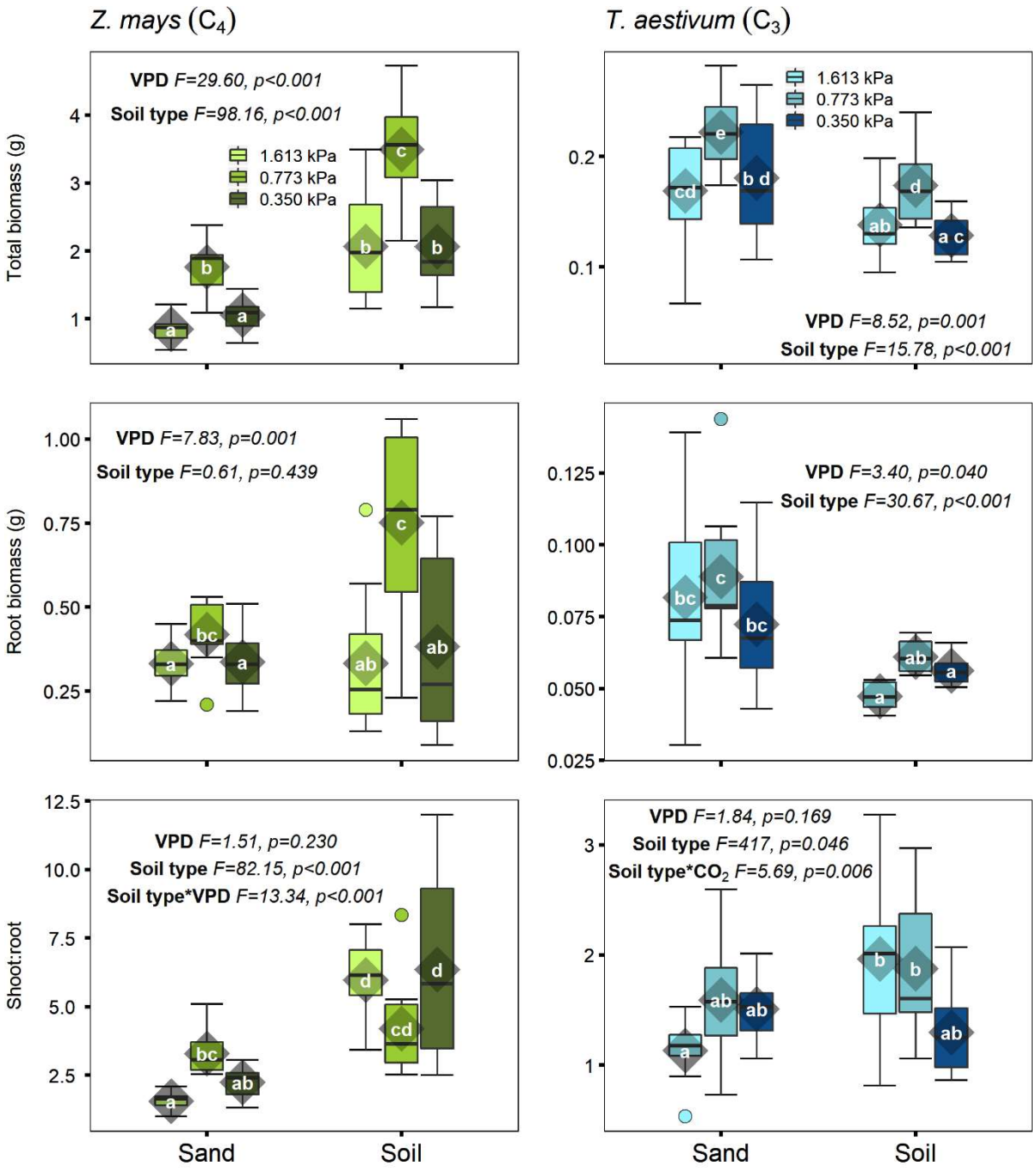
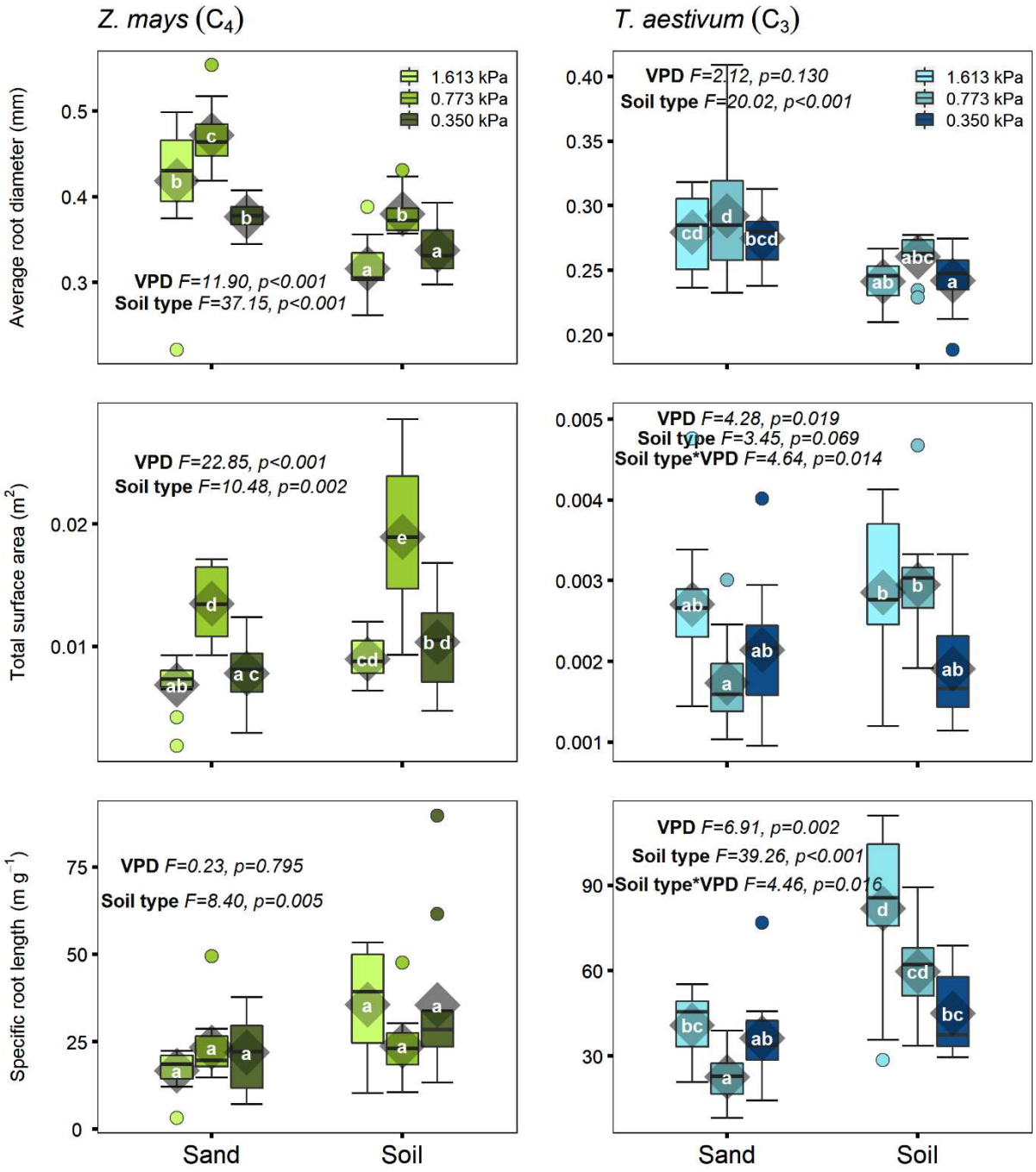


Figure 1



1503

1504 **Figure 2**

1505

1506

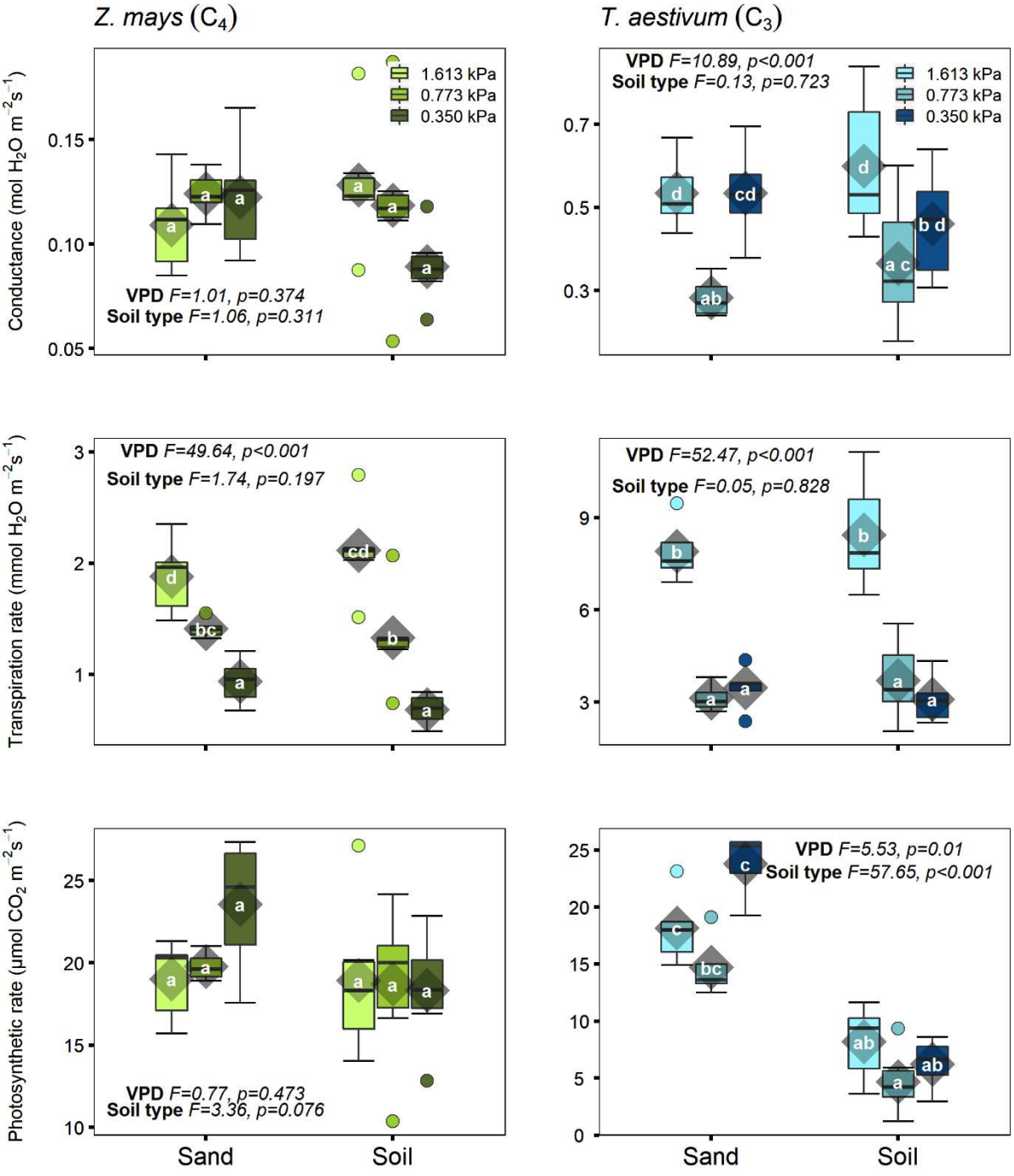
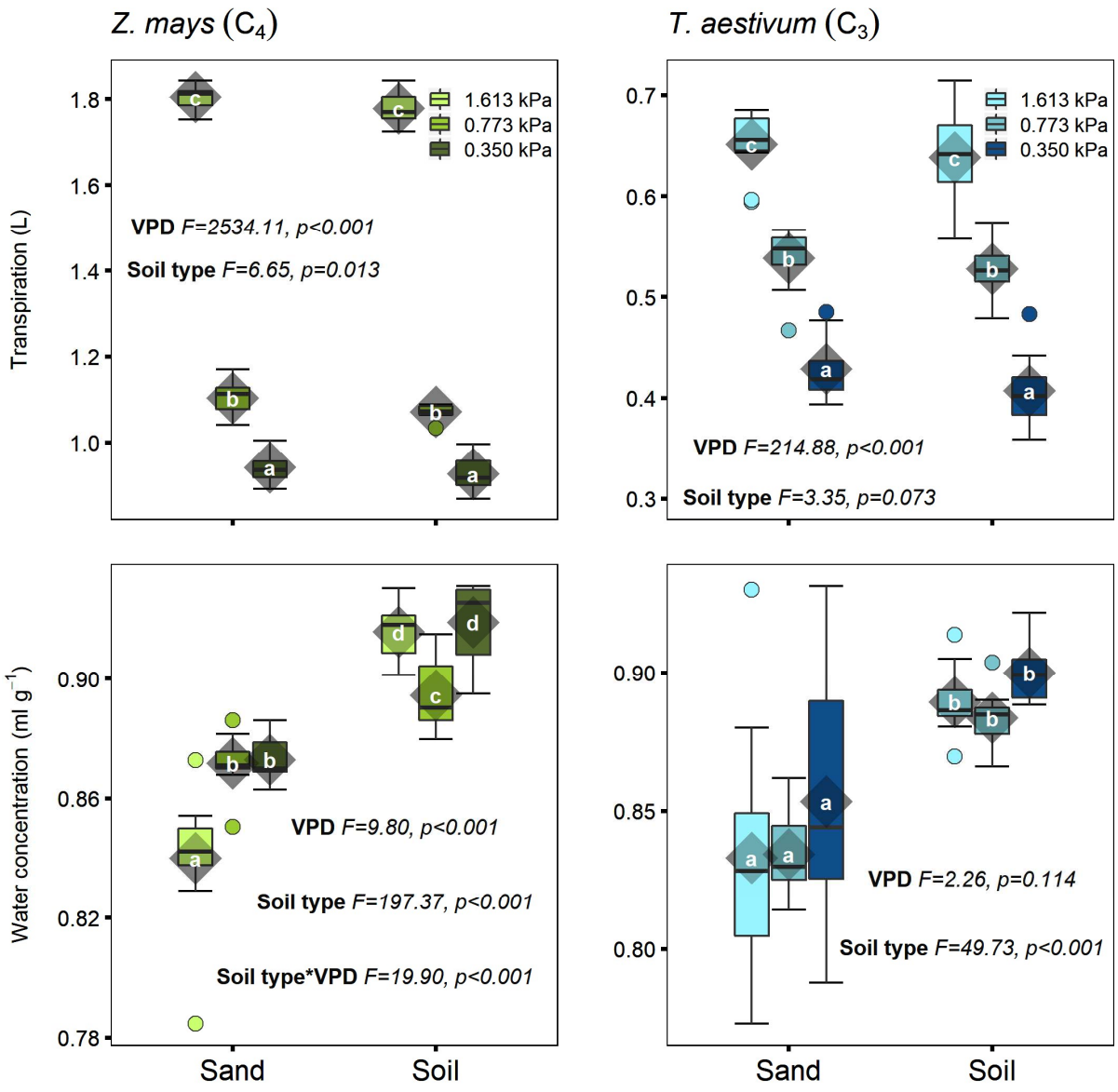

Z. mays (C4)
T. aestivum (C3)
1.613 kPa
0.773 kPa
0.350 kPa
Conductance (mol H2O m-2s-1)
VPD F=1.01, p=0.374
Soil type F=1.06, p=0.311
VPD F=10.89, p<0.001
Soil type F=0.13, p=0.723
Transpiration rate (mmol H2O m-2s-1)
VPD F=49.64, p<0.001
Soil type F=1.74, p=0.197
VPD F=52.47, p<0.001
Soil type F=0.05, p=0.828
Photosynthetic rate (µmol CO2 m-2s-1)
VPD F=0.77, p=0.473
Soil type F=3.36, p=0.076
VPD F=5.53, p=0.01
Soil type F=57.65, p<0.001
Sand
Soil

Figure 3



1510

1511 **Figure 4**

1512

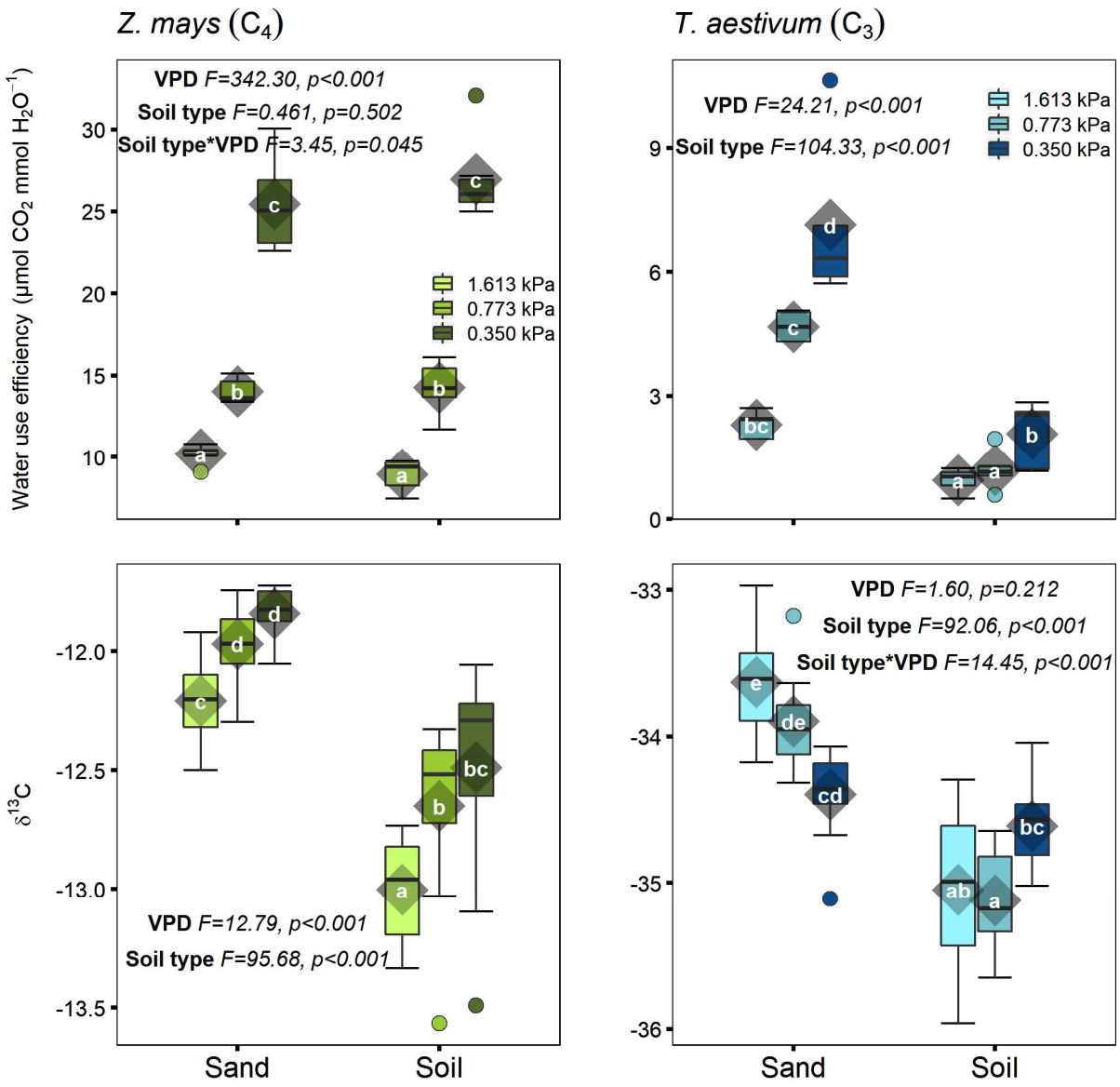
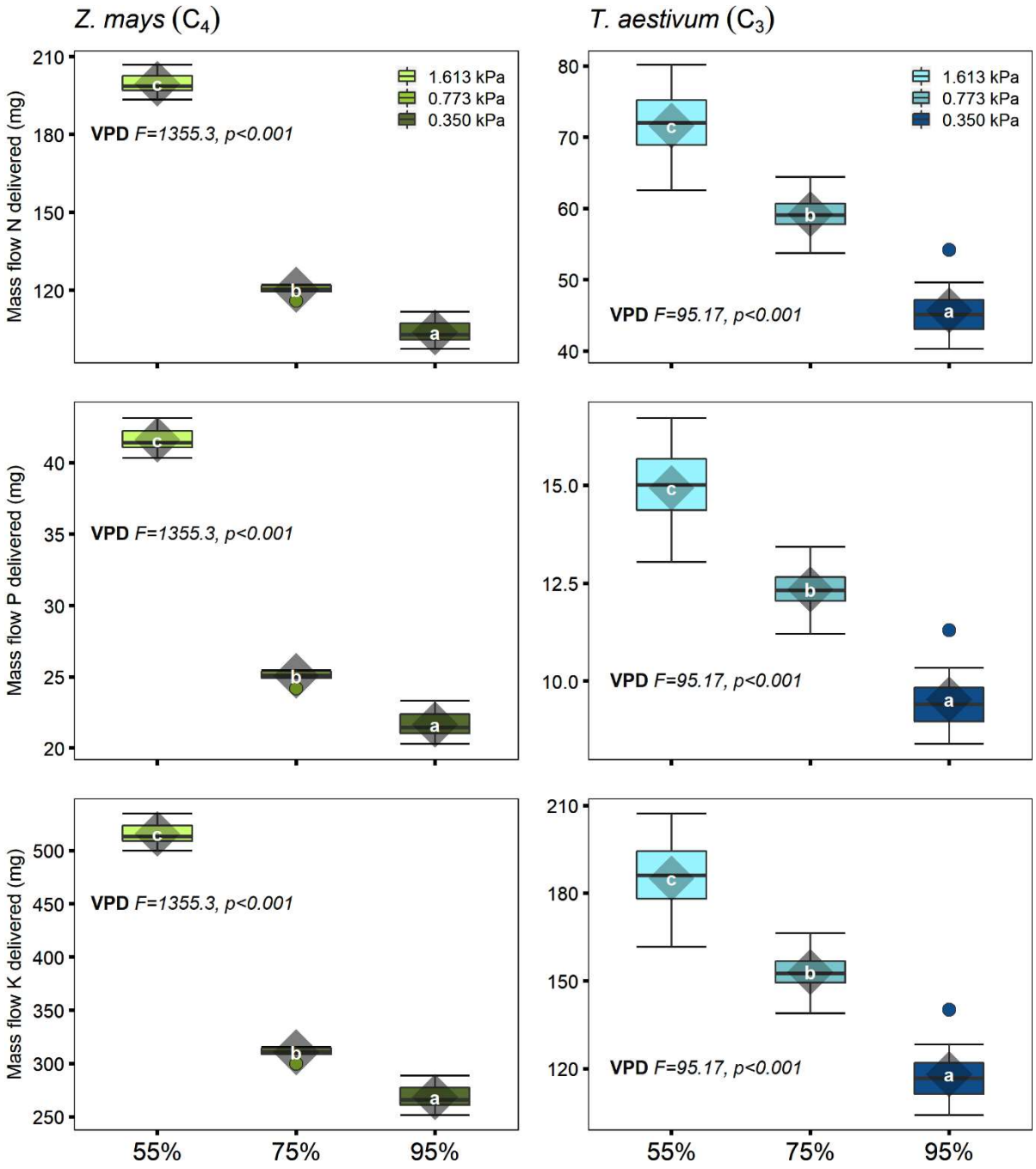

Z. mays (C4)
T. aestivum (C3)
Water use efficiency (µmol CO2 mmol H2O-1)
δ13C
VPD F=342.30, p<0.001
Soil type F=0.461, p=0.502
Soil type*VPD F=3.45, p=0.045
VPD F=24.21, p<0.001
Soil type F=104.33, p<0.001
1.613 kPa
0.773 kPa
0.350 kPa
VPD F=12.79, p<0.001
Soil type F=95.68, p<0.001
VPD F=1.60, p=0.212
Soil type F=92.06, p<0.001
Soil type*VPD F=14.45, p<0.001
Sand
Soil

Figure 5



1519

1520 **Figure 6**

1521

1522

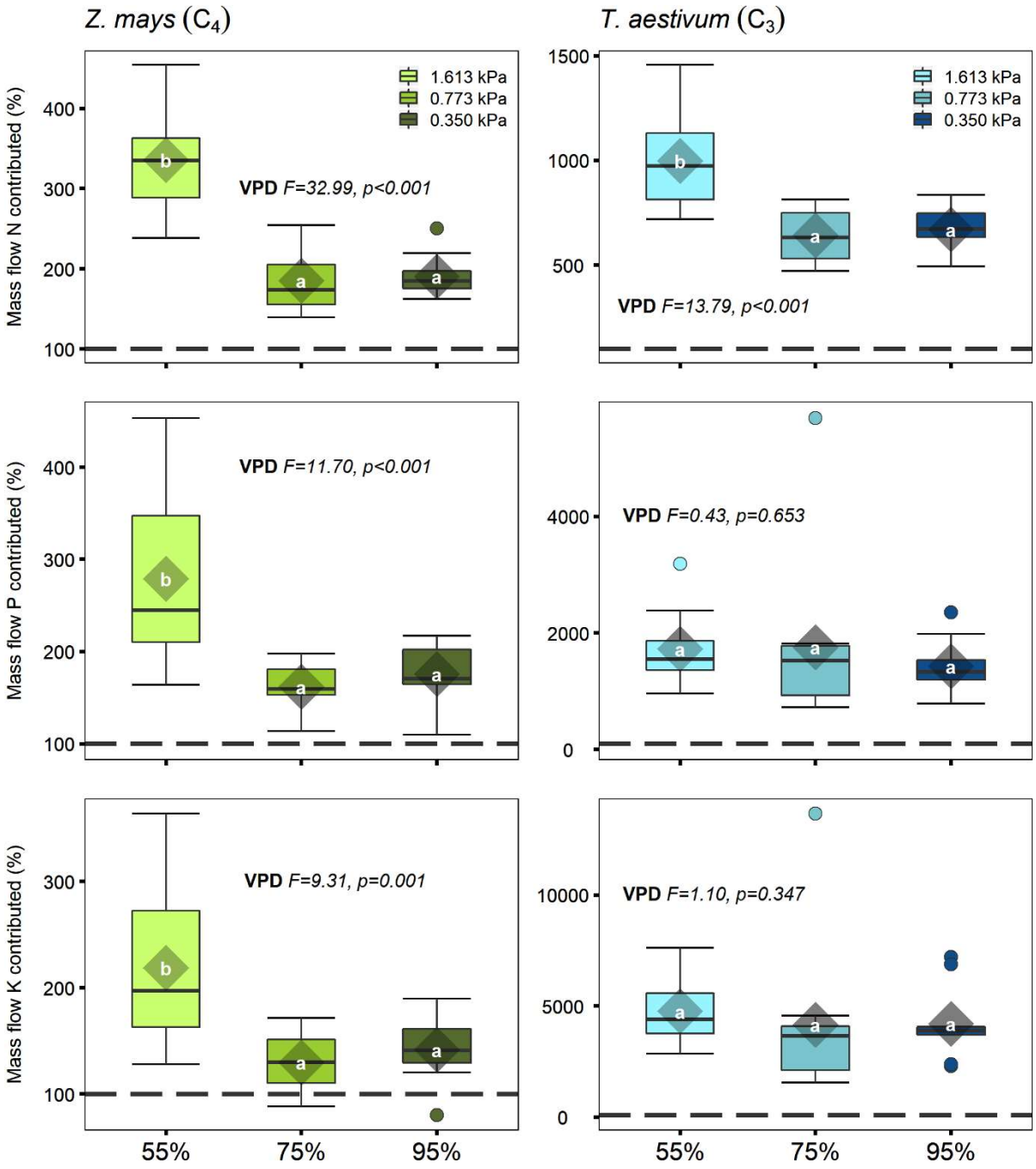

Z. mays (C4)
T. aestivum (C3)
1.613 kPa
0.773 kPa
0.350 kPa
Mass flow N contributed (%)
VPD F=32.99, p<0.001
VPD F=13.79, p<0.001
Mass flow P contributed (%)
VPD F=11.70, p<0.001
VPD F=0.43, p=0.653
Mass flow K contributed (%)
VPD F=9.31, p=0.001
VPD F=1.10, p=0.347
55%
75%
95%

Figure 7

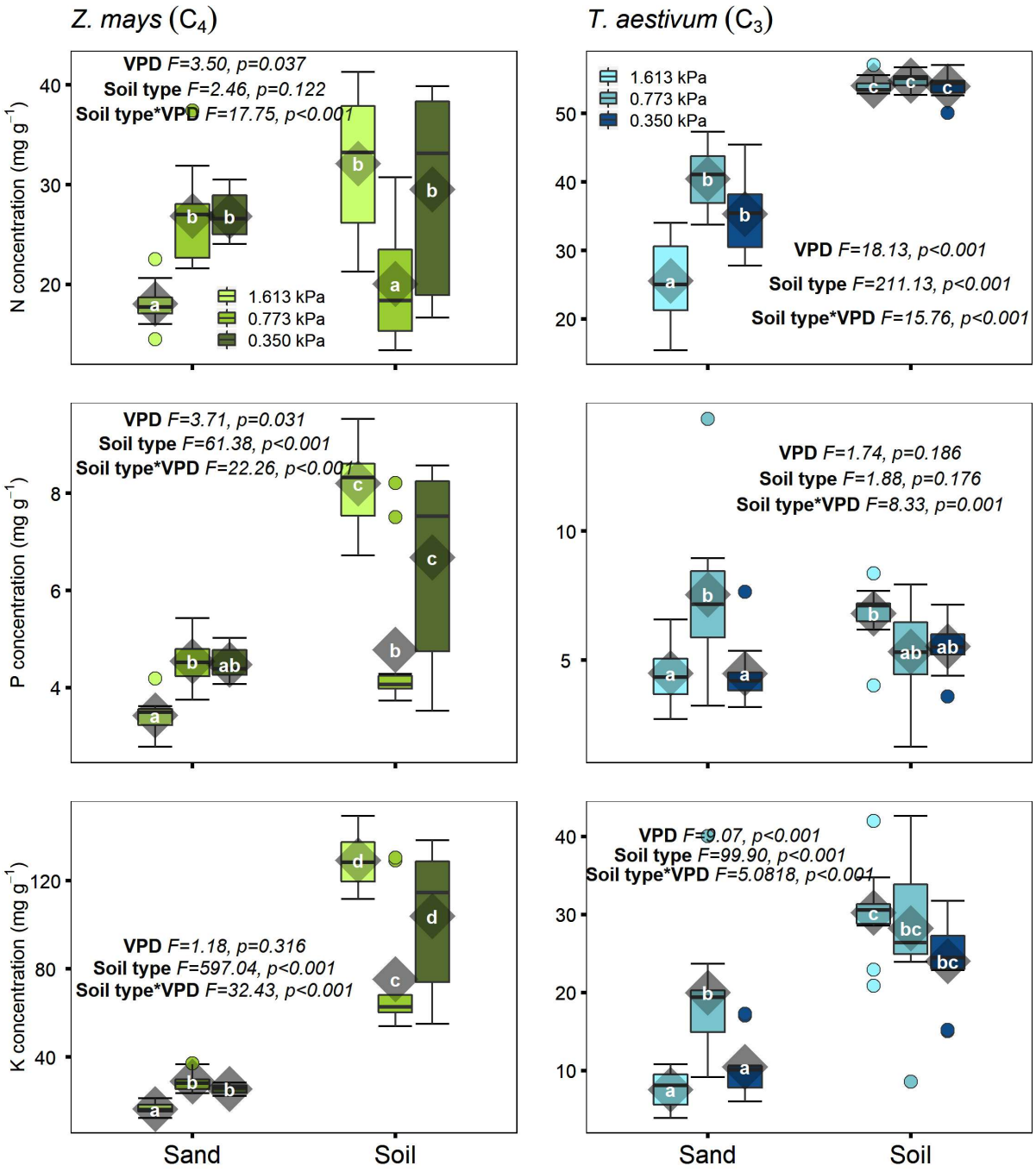

Z. mays (C4)
T. aestivum (C3)
N concentration (mg g-1)
P concentration (mg g-1)
K concentration (mg g-1)
VPD F=3.50, p=0.037
Soil type F=2.46, p=0.122
Soil type*VPD F=17.75, p<0.001
1.613 kPa
0.773 kPa
0.350 kPa
VPD F=18.13, p<0.001
Soil type F=211.13, p<0.001
Soil type*VPD F=15.76, p<0.001
VPD F=3.71, p=0.031
Soil type F=61.38, p<0.001
Soil type*VPD F=22.26, p<0.001
VPD F=1.74, p=0.186
Soil type F=1.88, p=0.176
Soil type*VPD F=8.33, p=0.001
VPD F=1.18, p=0.316
Soil type F=597.04, p<0.001
Soil type*VPD F=32.43, p<0.001
VPD F=9.07, p<0.001
Soil type F=99.90, p<0.001
Soil type*VPD F=5.0818, p<0.001
Sand
Soil

Figure 8

Chapter 4

General discussion and synthesis

New perspectives?

Many studies have reported decreases in the nutritional value of a range of socio-economically important crops globally when grown under conditions of elevated atmospheric CO_2 ('eCO_2') (Loladze, 2002; Taub, Miller & Allen, 2008; Mcgrath & Lobell, 2013; Zhu et al., 2018). These decreases apply to a range of macronutrients such as N, P, K and Ca, as well as micronutrients such as Fe, Mn, Cu and Zn (e.g. in Fig. 1).

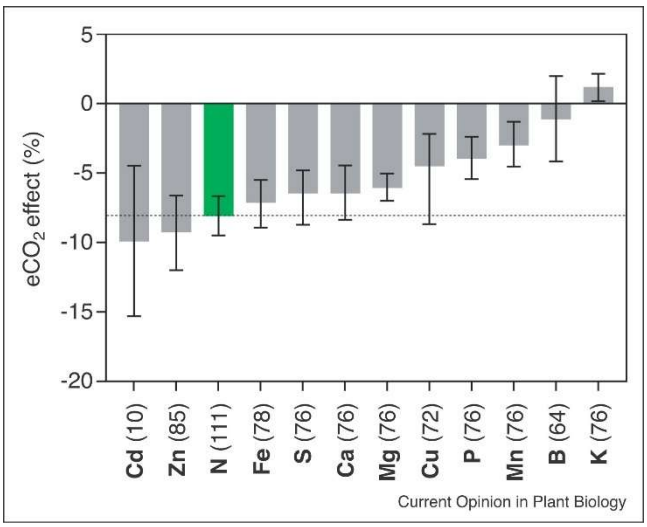


Figure 1: The effects of eCO_2 on wheat grain concentration of 12 different elements. Data are from a global meta-analysis on wheat (Broberg, Högy & Pleijel, 2017). The dashed horizontal line represents the effect on N for comparison. Figure obtained from Uddling et al. (2018).

Currently, it is estimated that 25% and 31% of World's population are iron and zinc-deficient respectively (Ezzati et al., 2004). This global malnutrition problem could be further exacerbated, since no decrease in atmospheric [CO_2] is expected before the end of the century, even under the best mitigation scenarios (Fisher, Nakicenovic & Alfsen, 2007). Several

hypotheses have been proposed for the decrease in nutritional value of important crops at eCO_2, from reduced root development, dilution effects due to enhanced carbohydrate production, downregulation of photosynthesis and reduced transpiration-driven mass delivery of nutrients (Taub & Wang, 2008). In Chapters 2 and 3, mass flow delivery of nutrients was reduced in maize and wheat plants by growing them at eCO_2 (Chapter 2), and by decreasing vapour pressure deficits ('VPD') (Chapter 3), in order to determine the importance of this process on nutrient delivery and acquisition in these two species. Reduced mass flow at eCO_2 is generally accepted as a cause for decreased nutritional value in C_3 plants, but C_4 plants remain understudied in that context, possibly due to their apparent lack of response to eCO_2. The findings from Chapter 2 reveal that reductions in mass flow at eCO_2 occur in both the C_3 crop (wheat) and the C_4 crop (maize), as a result of decreased stomatal conductance and transpiration rates. However, no change in the tissue nutrient concentrations was observed in neither species and therefore the hypothesis that eCO_2-induced reductions in mass flow lead to decreased nutritional value wheat and maize was not supported. The lack of attributed to the well-watered status of the soil treatments (70-100% field capacity), which may have allowed for mass flow and diffuses processes to interchangeably transport nutrients to their root systems, even though mass flow was decreased. The findings from Chapter 3 corroborate the findings from Chapter 2, whereby decreases in mass flow delivery of nutrients with increasing relative air humidity did not affect tissue nutrient concentrations in either species under well-watered conditions. Combining the findings from both chapters and from the literature, I first argue that there is not enough evidence to support the hypothesis that reductions in mass flow lead to decreased nutrient concentrations at the leaf level in wheat and maize plants under well-watered conditions. I then argue against the idea that the nutrient status of C_4 crops are generally less at risk of deleterious effects under conditions of elevated atmospheric [CO_2] (Myers et al., 2014; Uddling et al., 2018).

Importance of soil water status on effects of reduced mass flow at eCO_2 and low VPD

It could be argued that the lack of decrease in tissue nutrient concentrations in the maize and wheat plants grown at eCO_2 (Chapter 2) was due to 'pot effects'. Potted experiments, such as the ones conducted in Chapters 2 and 3, can firstly lead to different water dynamics compared to field conditions, while small soil volumes may allow for unrestricted access to nutrients by the roots, regardless of whether mass flow is reduced or not (Lieffering et al., 2004; Mcgrath & Lobell, 2013). However, in a recent meta-analysis that included data from free-air CO_2 (FACE) experiments as well as from chamber experiments, decreases of a range of nutrients was found in crop species such as wheat or rice grown at eCO_2 (Myers et al., 2014). This potentially suggests that 'pot effects' may not be responsible for the unchanged nutrient concentrations of the maize and wheat plants grown at eCO_2. It was instead suggested that the well-watered status of the soil treatments (kept between 70-100% field capacity) may have allowed for the adequate delivery of nutrients by mass flow to the rhizosphere, even when transpiration was decreased (i.e. at eCO_2). In their meta-analysis, Mcgrath & Lobell (2013) state that the importance of mass flow can vary depending soil water content and soil solution nutrient concentrations. Thus, in well-watered substrates, with high nutrient concentrations in the soil solution, a lesser amount of water displaced (e.g. by mass flow) may nonetheless supply adequate amounts of nutrients to the rhizosphere. I thus argue that when investigating the effects of reduced transpiration and mass flow on the nutrient concentrations in plants, it is important to consider how the nutrient and water status of the soil may mediate the importance of mass flow for delivery of nutrients. For example, in a study done by del Pozo et al. (2007), decreases in N concentration in wheat plants grown at eCO_2 were attributed to a decrease in mass flow of N, as a result of decreased transpiration. The wheat plants in this study were exposed to soil water conditions that replicated water availability in a rainfed agricultural system, in Salamanca, Spain (i.e. not watered to field capacity). The findings from the above

study thus demonstrates that reductions in mass flow under conditions of limited soil water availability may indeed lead to decreased nutrient concentrations.

Under well-watered conditions in the eCO_2 experiment (Chapter 2), mass flow contribution of most nutrients to tissue content in both the maize and wheat plants was nonetheless significantly decreased at eCO_2, and yet their nutrient status remained unchanged. In that experiment, mass flow delivery of nutrients was calculated as the cumulative water use of a plant throughout its lifetime, multiplied by the concentration of that element in soil solution (Barber, 1995). Oyewole, Inselsbacher & Näsholm (2014) argue that challenges associated with this method are that they do not take into account spatial and temporal variations in soil water content, as well as potential interactions between mass flow and diffusion. Diffusive processes happen due to concentration gradients in and about the rhizosphere as a result of active uptake by root of plants (Barber, 1995). Even when transpiration and mass flow are reduced, movement of nutrients to the rhizosphere may thus be facilitated by diffusion under well-watered conditions. The linear regressions between amount of nutrients delivered by mass flow and tissue nutrient contents in the eCO_2 experiment showed higher N, P and K contents at the intercepts (i.e. when 0 mg of N, P and K was delivered by mass flow) in both maize and wheat. This strongly suggests that diffusive processes may have compensated for reductions in mass flow at eCO_2. In agreement with these findings, Van Vuuren et al. (1997) found that diffusive processes compensated for reductions in mass flow at eCO_2 in wheat plants grown in wet conditions, allowing them to maintain higher foliar N, compared to the ones grown at eCO_2 under dry conditions. Thus, in the latter study, the negative effects of reductions in transpiration-driven mass flow at eCO_2 were alleviated under adequate water supply. McDonald et al. (2002) made a similar observation in *Populus deltoides* trees grown at eCO_2 under varying relative air humidity. In that study, it was stated that diffusive processes with

high soil moisture in the high humidity treatments compensated for the eCO_2-induced reductions in mass flow for the delivery of NO_3^- to the trees.

Since high water availability is a condition that is not always met under field conditions (Marschner & Rengel, 2011), especially in rainfed agricultural systems (Lobell et al., 2014), eCO_2-induced reductions in transpiration may severely impact the nutritional value of many important crops globally. Decreases in the nutrient concentrations of plants exposed to eCO_2 are often attributed to a range of different mechanisms (Taub & Wang, 2008; Mcgrath & Lobell, 2013; Myers et al., 2014), which seem to vary in importance depending on environmental factors, as well as on species. For example, under well-watered conditions, photosynthesis is more likely to be stimulated in C_3 plant species at eCO_2 (Ainsworth & Rogers, 2007), and this can potentially make them more prone to dilution effects compared to C_4 plants species. The results from Chapter 2 confirm this assertion, since it was found that photosynthesis was greatly stimulated by eCO_2 in wheat compared to the maize plants, under well-watered conditions. In the case of reductions in transpiration driven-mass flow, the results from the eCO_2 Chapter, combined with findings the literature, show that the effects of this particular mechanism at eCO_2 can be strongly dependent on factors such as soil water status, soil texture and soil nutrient availability (Van Vuuren et al., 1997; del Pozo et al., 2007; McDonald et al. 2002; Oliveira et al., 2010; Mcgrath & Lobell, 2013).

C_3 versus C_4 crops in the context of mass flow reductions

Many studies have argued that C_4 plants are little affected by eCO_2 (Wong, 1979; Reeves et al., 1994; Uddling et al., 2018) due to their photosynthetic mechanism usually being saturated at ambient CO_2 (Ziska & Bunce, 1997). However, it has been shown that growth of C_4 plants can be stimulated under drought conditions at eCO_2 due to improved water-use efficiency as a result of decreased stomatal conductance and sustained intercellular [CO_2] (Ainsworth &

Rogers, 2007; Allen et al., 2011). Additionally, the results from Chapters 2 and 3 show that decreases in stomatal conductance and transpiration can occur in both the C_3 and the C_4 species. Thus, decreases in mass flow at eCO_2 and at low VPD occurred regardless of photosynthetic pathway, results which are in agreement with previous findings the literature (Wong, 1979; Tanner & Beevers, 1990; Loladze, 2002; Ainsworth & Long, 2005). Under conditions of limited soil water availability and eCO_2, reported increases in biomass accumulation (Ainsworth & Rogers, 2007), combined with reductions in stomatal conductance and transpiration (as shown by the results in Chapter 2), may in fact put C_4 crops equally at risk as C_3 crops in terms decreases in nutritional value under changing climatic conditions (e.g. eCO_2), as a result of decreased mass flow delivery of nutrients in addition to dilution effects. This is of particular concern because both wheat and maize are major sources of proteins and carbohydrates for a great proportion of the global population (Myers et al., 2014; Uddling et al., 2018) and continued increased in atmospheric [CO_2] may exacerbate the already existing global malnutrition problem.

While mass flow delivered nutrients in excess of physiological requirements in both maize and wheat at all CO_2 and VPD levels respectively in both experiments (attributed to well-watered conditions), the total amount of nutrients delivered by mass flow was nonetheless significantly reduced at eCO_2 and with decreasing VPD. Thus, under conditions of low soil water availability and nutrient availability, reductions in transpiration (i.e. at eCO_2 or low VPD) will most likely reduce the amount of nutrients available in the rhizosphere for uptake by the plants (Mortensen, 1987). In addition, while transpiration tends to generally affect delivery of nutrients, it has been suggested than uptake across the root membrane could also be reduced when transpiration is decreased due to decreased element flux through the root apoplasm (Marschner & Marschner, 2012). Furthermore, movement of nutrients from the root to the shoot can also be impeded as a result of decreased xylem water movement when transpiration

is reduced, as shown by the decrease in foliar N in aspen trees grown under humid conditions (Tullus et al., 2012). The findings from both Chapters 2 and 3, in combination with findings from the literature, thus demonstrate the importance of taking into consideration plant transpiration-driven water fluxes both in the soil, and within the plants, in tandem with other metabolic and physiological changes (Taub & Wang, 2008), for the preservation of the nutritional value and yield of socio-economically important C_3 and C_4 crops under changing climatic conditions.

Moving forward

While nutrient or protein concentrations of the edible parts of the maize and wheat plants were not investigated at the different CO_2 or VPD levels in the present, it is well established that nutrients such as N are mostly translocated from the vegetative parts of these plants during grain filling (Papakosta & Gagianas, 1991; Mae, 1997; Ntanos & Koutroubas, 2002). For example, 40% of the N required for grain development in wheat was translocated from the leaves, with the other 60% coming from the stem, glumes and roots (Simpson, Lambers & Dalling, 1983). In maize, it has been shown that 56% of the total N translocated to the grain was from the aboveground vegetative parts (Beauchamp, Kannenberg & Hunter, 1976). Thus, studies investigating changes in foliar nutrients under conditions of reduced mass flow, such as the present one, can therefore inform agricultural management practices on how to maintain, mitigate, or improve the nutritional status of these two crops under changing climatic conditions.

Adequate water availability and increased fertiliser supply can potentially alleviate the negative effects of eCO_2 on the nutrient status of maize and wheat (Kimball, 2006; Bunce, 2014), but the use of these strategies is not always feasible, due to associated costs and environmental problems. Water is often one of the most limiting resource in cultivated systems

globally (Gerten et al., 2004; Mu et al., 2007). In addition, with the changing climate, global increases in temperature are expected, while the opposite is expected for precipitation in many regions where socio-economically important crops are grown (Giorgi et al., 2001; Kling et al., 2003; Weltzin et al., 2003), thereby increasing the risk of drought events. Furthermore, in many cultivated systems, increased application of fertiliser is not always possible due to cost constraints (Wallace & Knausenenberger, 1997), while excessive fertiliser inputs may lead to a whole range of environmental problems, the most common one being eutrophication (Galloway et al., 2008).

Moreover, water being a limited resource from crop production, there has been a drive to develop more water-use efficient genotypes in the last decade (Fletcher, Sinclair & Allen, 2007; Gholipoor et al., 2010; Jyostna Devi, Sinclair & Vadez, 2010; Yang et al., 2012), and it is possible that this may exacerbate the negative effects of eCO_2-induced reductions in mass flow on the nutrient status of these crops. It has been shown that decreased transpiration in water-use efficient cultivars of many important crops under conditions of eCO_2, high temperatures, or high VPD, increases soil moisture conservation in early growth stages, thereby leading to increased biomass accumulation as a result of more water being available in the late growing stages (Hunsaker et al., 2000; Leakey et al., 2006). Based on the findings from the study done by Van Vuuren et al. (1997), I argue that decreased transpiration and reduced soil water movement in water-use efficient genotypes may lead to inadequate supply of nutrients by mass flow to the root system of these crops, thereby leading to decreased tissue nutrient content, under conditions of low soil water availability and increasing global atmospheric $[CO_2]$. Additionally, increased biomass accumulation is often measured in limited water environments at eCO_2 in C_4 crops such as maize and sorghum (Reeves et al., 1994; Ainsworth & Rogers, 2007). Decreases in the nutritional value may be even further exacerbated, by a

1719 combination of reduced mass flow and dilution effects, in more water-use efficient cultivars of
1720 these C_4 crops.

1721 Moving forward, in addition to breeding water-use efficient cultivars, a possible way
1722 to mitigate the impacts of increasing atmospheric [CO_2] on the quality and yield of important
1723 crops globally can be to breed for cultivars of C_3 and C_4 crops that display reduced sensitivity
1724 to eCO_2 (Myers et al., 2014). For example, reduced stomatal closure at eCO_2 could be achieved
1725 by genetically modifying the CO_2 cellular sensing mechanism of plants. Such modifications
1726 include lowering abscisic acid (ABA) synthesis or modifying the expression patterns of
1727 carbonic anhydrases (CA) under conditions of eCO_2 (Engineer et al., 2016). Both ABA and
1728 CA function in mediating stomatal responses to CO_2 (Jones & Mansfield, 1970; Stimler, Berry
1729 & Yakir, 2012).

1730

References

Acevedo, E., Hsiao, T.C. & Henderson, D.W. 1971. Immediate and Subsequent Growth Responses of Maize Leaves to Changes in Water Status. *Plant Physiology*. 8(5):631–636.

Adams, P. & Hand, D.J. 1993. Effects of humidity and Ca level on dry-matter and Ca accumulation by leaves of cucumber (*Cucumis sativus* L.) . *Journal of Horticultural Science*. 68(5):767–774.

Ainsworth, E.A. & Long, S.P. 2005. What have we learned from 15 years of free-air CO2enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. *New Phytologist*. 165(2):351–372.

Ainsworth, E.A. & Rogers, A. 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: Mechanisms and environmental interactions. *Plant, Cell and Environment*. 30(3):258–270.

Allen, L.H., Kakani, V.G., Vu, J.C.V. & Boote, K.J. 2011. Elevated CO_2 increases water use efficiency by sustaining photosynthesis of water-limited maize and sorghum. *Journal of Plant Physiology*. 168(16):1909–1918.

Barber, S.A. 1995. *Soil nutrient bioavailability: a mechanistic approach*. John Wiley & Sons.

Barber, S.A. & Cushman, J. 1981. Nitrogen uptake model for agronomic crops. In *Modeling waste water renovation-land treatment*. New York, NY, USA: Wiley-Interscience. 382–409.

Barber, S.A., Walker, J.M. & Vasey, E.H. 1963. Mechanisms for Movement of Plant Nutrients from Soil and Fertilizer to Plant Root. *Journal of Agricultural and Food*

1754 *Chemistry*. 11:204–207.

1755 BassiriRad, H., Reynolds, J.F., Virginia, R.A. & Brunelle, M.H. 1997. Growth and Root NO_3^-
1756 and PO_4^{3-} Uptake Capacity of Three Desert Species in Response to Atmospheric CO_2
1757 Enrichment. *Australian Journal of Plant Physiology*. 24:353–358.

1758 BassiriRad, H., Gutschick, V.P. & Lussenhop, J. 2001. Root system adjustments: Regulation
1759 of plant nutrient uptake and growth responses to elevated CO_2. *Oecologia*.
1760 126(3):305–320.

1761 Baysdorfer, C. & Robinson, J.M. 1985. Sucrose and Starch Synthesis in Spinach Plants
1762 Grown under Long and Short Photosynthetic Periods. *Plant Physiology*. 79(3):838–
1763 842.

1764 Beauchamp, E.G., Kannenberg, L.W. & Hunter, R.B. 1976. Nitrogen Accumulation and
1765 Translocation in Corn Genotypes Following Silking 1 . *Agronomy Journal*.
1766 68(2):418–422.

1767 Bernacchi, C.J., Kimball, B.A., Quarles, D.R., Long, S.P. & Ort, D.R. 2006. Decreases in
1768 Stomatal Conductance of Soybean under Open-Air Elevation of [CO_2] Are Closely
1769 Coupled with Decreases in Ecosystem Evapotranspiration. *Plant Physiology*.
1770 143(1):134–144.

1771 Berntson, G. M.; Woodward, F.I. 1992. The Root System Architecture and Development of
1772 *Senecio vulgaris* in Elevated CO_2 and Drought. *Functional Ecology*. 6(3):324–333.

1773 Berntson, G. 1994. Modeling Root Architecture - Are There Tradeoffs Between Efficiency
1774 and Potential of Resource Acquisition. *New Phytologist*. 127(3):483–493.

1775 Berntson, G.M. 1944. Modelling Root Architecture : Are There Tradeoffs between Efficiency
1776 and Potential of Modelling tradeoffs root between architecture : efficiency and are

there potential of resource acquisition? *New Phytologist*. 127(3):483–493.

Berntson, G.M. & Bazzaz, F.A. 1996. Belowground positive and negative feedbacks on CO_2 growth enhancement. *Plant and Soil*. 187(2):119–131.

Bloom, A.J., Caldwell, R.M., Finazzo, J., Warner, R.L. & Weissbart, J. 1989. Oxygen and Carbon Dioxide Fluxes from Barley Shoots Depend on Nitrate Assimilation. *Plant Physiology*. 91(1):352–356.

Bloom, A.J., Smart, D.R., Nguyen, D.T. & Searles, P.S. 2002. Nitrogen assimilation and growth of wheat under elevated carbon dioxide. *Proceedings of the National Academy of Sciences*. 99(3):1730–1735.

Bowes, G. 1993. Facing the Inevitable : Plants and Increasing Atmospheric CO_2. *Annual review of plant physiology and plant molecular biology*. 44:309–332.

Bowman, W.D., Hubick, K.T., Caemmerer, S. Von & Farquhar, G.D. 1989. Short-Term Changes in Leaf Carbon in Salt- and Water-Stressed C_4 Grasses. 90(1):162–166.

Broberg, M.C., Högy, P. & Pleijel, H. 2017. CO_2 -induced changes in wheat grain composition: Meta-analysis and response functions. *Agronomy*. 7(2):1–18.

Brouder, S.M. & Volenec, J.J. 2008. Impact of climate change on crop nutrient and water use efficiencies. *Physiologia Plantarum*. 133(4):705–724.

Brück, H., Payne, W.A. & Sattelmacher, B. 2000. Effects of phosphorus and water supply on yield, transpirational water-use efficiency, and carbon isotope discrimination of pearl millet. *Crop Science*. 40(1):120–125.

Bunce, J.A. 1982. Low humidity effects on photosynthesis in single leaves of C_4 plants. *Oecologia*. 54(2):233–235.

1799 Bunce, J.A. 1983. Differential Sensitivity to Humidity of Daily Photosynthesis in the Field C_3
1800 and C_4 Species. *Oecologia*. 57(1):262–265.

1801 Bunce, J.A. 1984. Effects of humidity on photosynthesis. *Journal of Experimental Botany*.
1802 35(9):1245–1251.

1803 Bunce, J.A. 1993. Effects of doubled atmospheric carbon dioxide concentration on the
1804 responses of assimilation and conductance to humidity. *Plant, Cell and Environment*.
1805 16:189–197.

1806 Bunce, J.A. 2014. Corn Growth Response to Elevated CO_2 Varies with the Amount of
1807 Nitrogen Applied. *American Journal of Plant Sciences*. 05(03):306–312.

1808 Cakmak, I., Pfeiffer, W.H. & McClafferty, B. 2010. REVIEW: Biofortification of Durum
1809 Wheat with Zinc and Iron. *Cereal Chemistry*. 87(1):10–20.

1810 Cernusak, L.A., Goldsmith, G.R., Arend, M. & Siegwolf, R.T.W. 2019. Effect of vapor
1811 pressure deficit on gas exchange in wild-type and abscisic acid–insensitive plants.
1812 *Plant Physiology*. 181(4):157–1586.

1813 Chapagain, T. 2014. Root Architecture Variation in Wheat and Barley Cultivars. *American*
1814 *Journal of Experimental Agriculture*. 4(7):849–856.

1815 Chapin, F.S. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and*
1816 *Systematics*. 11:233-260

1817 Chapman, N., Miller, A.J., Lindsey, K. & Whalley, W.R. 2012. Roots, water, and nutrient
1818 acquisition: Let's get physical. *Trends in Plant Science*. 17(12):701–710.

1819 Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L.,
1820 Carvalho, I., Faria, T., Pinheiro, C. 2002. How plants cope with water stress in the
1821 field. Photosynthesis and growth. *Annals of Botany*. 89:907–916.

1822 Ciais, P., C. Sabine, G. Bala, L. Bopp, V. Brovkin, J. Canadell, A. Chhabra, R. DeFries, J.
1823 Galloway, M. Heimann, C.

1824 Jones, C. Le Quéré, R.B. Myneni, S. Piao and P. Thornton 2013: Carbon and Other
1825 Biogeochemical Cycles. In: *Climate Change 2013: The Physical Science Basis.*
1826 *Contribution of Working Group I to the Fifth Assessment Report of the*
1827 *Intergovernmental Panel on Climate Change*. Stocker, T.F., D. Qin, G.-K. Plattner,
1828 M. Tignor, S.K. Allen, J.Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley,
1829 Eds. Cambridge University Press, Cambridge, United Kingdom and New York, NY,
1830 USA

1831 Comas, L.H., Bouma, T.J. & Eissenstat, D.M. 2002. Linking root traits to potential growth
1832 rate in six temperate tree species. *Oecologia*. 132(1):34–43.

1833 Comstock, J. & Ehleringer, J. 1993. Stomatal response to humidity in common bean
1834 (*Phaseolus vulgaris*): Implications for maximum transpiration rate, water-use
1835 efficiency and productivity. *Australian Journal of Plant Physiology*. 20(6):669–691.

1836 Condon, A.G., Richards, R.A. & Farquhar, G.D. 1993. Relationships between carbon isotope
1837 discrimination, water use efficiency and transpiration efficiency for dryland wheat.
1838 *Australian Journal of Agricultural Research*. 44(8):1693–1711.

1839 Condon, A.G., Richards, R.A., Rebetzke, G.J. & Farquhar, G.D. 2002. Improving Intrinsic
1840 Water-Use Efficiency and Crop Yield. *Crop Science*. 42(February):122–131.

1841 Conroy, J. 1992. Influence of Elevated Atmospheric CO_2 Concentrations on Plant Nutrition.
1842 *Australian Journal of Botany*. 40(5):445.

1843 Cornic, G. 2000. Drought stress inhibits photosynthesis dy decreasing stomatal aperture - not
1844 by affecting ATP synthesis. *Trends in Plant Science*. 5:187–188.

1845 Cousins, A.B., Adam, N.R., Wall, G.W., Kimball, B.A., Pinter, P.J., Ottman, M.J., Leavitt,
1846 S.W. & Webber, A.N. 2003. Development of C_4 photosynthesis in sorghum leaves
1847 grown under free-air CO_2 enrichment (FACE). *Journal of Experimental Botany*.
1848 54(389):1969–1975.

1849 Cramer, M.D. & Hawkins, H.J. 2009. A physiological mechanism for the formation of root
1850 casts. *Palaeogeography, Palaeoclimatology, Palaeoecology*. 274(3–4):125–133.

1851 Cramer, M.D., Hoffmann, V. & Verboom, G.A. 2008. Nutrient availability moderates
1852 transpiration in Ehrharta calycina. *New Phytologist*. 179(4):1048–1057.

1853 Cramer, M.D., Hawkins, H.-J. & Verboom, G.A. 2009. The importance of nutritional
1854 regulation of plant water flux. *Oecologia*. 161(1):15–24.

1855 Crawford, N.M. & Glass, A.D.M. 1998. Molecular and physiological aspects of nitrate
1856 uptake in plants. *Trends in Plant Science*. 3(10):389–395.

1857 Cure, J.D. & Acock, B. 1986. Crop responses to carbon dioxide doubling: a literature survey.
1858 *Agricultural and Forest Meteorology*. 38(1–3):127–145.

1859 Davey, P.A., Parsons, A.J., Atkinson, L., Wadge, K. & Long, S.P. 1999. Does photosynthetic
1860 acclimation to elevated CO_2 increase photosynthetic nitrogen-use efficiency ? A study
1861 of three native UK grassland species in open-top chambers. *Environmental Research*.
1862 13(1):21–28.

1863 Drake, B.G., Gonzàlez-Meler, M.A. & Long, S.P. 1997. MORE EFFICIENT PLANTS: A
1864 Consequence of Rising Atmospheric CO_2? . *Annual Review of Plant Physiology and*
1865 *Plant Molecular Biology*. 48(1):609–639.

1866 Ehleringer, J.R., Cerling, T.E. & Helliker, B.R. 1997. C_4 photosynthesis, atmospheric CO_2 ,
1867 and climate. *Oecologia*. 112(3):285–299.

1868 Eng, R.Y.N., Tsujita, M.J. & Grodzinski, B. 1985. The effects of supplementary HPS lighting
1869 and carbon dioxide enrichment on the vegetative growth, nutritional status and
1870 flowering characteristics of *Chrysanthemum morifolium* Ramat. *Journal of*
1871 *Horticultural Science*. 60(3):389–395.

1872 Engineer, C., Hashimoto-Sugimoto, M., Negi, J., Israelsson-Nordstrom, M., Azoulay-
1873 Shemer, T., Rappel, W.-J., Iba, K. & Schroeder, J. 2016. CO_2 sensing and CO_2
1874 regulation of stomatal conductance: advances and open questions. *Trends in Plant*
1875 *Science*. 21(1):16–30.

1876 Erbs, M., Manderscheid, R., Hüther, L., Schenderlein, A., Wieser, H., Dänicke, S. & Weigel,
1877 H.J. 2014. Free-air CO_2 enrichment modifies maize quality only under drought stress.
1878 *Agronomy for Sustainable Development*. 35(1):203–212.

1879 Erhardt, J.F. 1978. Unlcrsmhungcn iibcr Hydalhodcn. Technische Universitat, Munchen.

1880 Evans, L.T. 1993. *Crop Evolution, Adaptation, and Yield*. Cambridge University Press.

1881 Evans, R.D. 2001. Physiological mechanisms influencing plant nitrogen isotope composition.
1882 *Trends in Plant Science*. 6(3):121–126.

1883 Evans, J. & Seemann, J.R.. 1989. The allocation of protein nitrogen in the photosynthetic
1884 apparatus: Costs, consequences, and control. Photosynthesis. 183-205.

1885 Ezzati, M., Lopez, A.D., Rodgers, A. & Murray, C.J.L. 2004. Comparative quantification of
1886 health risks: global and regional burden of diseases attributable to selected major
1887 risks. *Comparative Quantification of Health Risks: Global and Regional Burden of*
1888 *Diseases Attributable to Selected Major Risk Factors*.

1889 Fangmeier, A., De Temmerman, L., Mortensen, L., Kemp, K., Burke, J., Mitchell, R., Van
1890 Oijen, M. & Weigel, H.J. 1999. Effects on nutrients and on grain quality in spring

wheat crops grown under elevated CO_2 concentrations and stress conditions in the European, multiple-site experiment “ESPACE-wheat”. *European Journal of Agronomy*. 10(3–4):215–229.

Fangmeier, A., Chrost, B. & Ho, P. 2000. CO_2 enrichment enhances flag leaf senescence in barley due to greater grain nitrogen sink capacity. *Environmental and Experimental Botany*. 44:151–164.

Fangmeier, A., De Temmerman, L., Black, C., Persson, K. & Vorne, V. 2002. Effects of elevated CO_2 and/or ozone on nutrient concentrations and nutrient uptake of potatoes. *European Journal of Agronomy*. 17(4):353–368.

FAO. (2013). Food and Agriculture Organization of the United Nations, FAOSTAT database. Rome: 703 Food and Agriculture Organization of the United Nations. URL http://faostat.fao.org/

Farquhar, G.D., O’Leary, M.H. & Berry, J.A. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*. 9(2):121–137.

Farquhar, G.D., Hubick, K.T., Condon, A.G. & Richards, R.A. 1989. Carbon Isotope Fractionation and Plant Water-Use Efficiency. *Stable Isotopes in Ecological Research*. 21–40.

Feng, Z., Rütting, T., Pleijel, H., Wallin, G., Reich, P.B., Kammann, C.I., Newton, P.C., Kobayashi, K., Luo, Y. and Uddling, J. 2015. Constraints to nitrogen acquisition of terrestrial plants under elevated CO2. *Global Change Biology*. 21:3152-3168.

Fernandez, M.C. & Rubio, G. 2015. Root morphological traits related to phosphorus-uptake efficiency of soybean, sunflower, and maize. *Journal of Plant Nutrition and Soil*

Science. 178(5):807–815.

Fisher, B.S., Nakicenovic, N. & Alfsen, K. 2007. Issues related to mitigation in the long term context. In *Climate change 2007: mitigation contribution of working group III to the Fourth Assessment Report of the Inter-governmental Panel on Climate Change*. B. Metz, O.R. Davidson, P.R. Bosch, R. Dave, & L.A. Meyer, Eds. Cambridge: Cambridge University Press. 169–250.

Fitter, A.H. 1987. An architectural approach to the comparative ecology of plant root systems. *New Phytologist*. 106:61–77.

Fletcher, A.L., Sinclair, T.R. & Allen, L.H. 2007. Transpiration responses to vapor pressure deficit in well watered "slow-wilting" and commercial soybean. *Environmental and Experimental Botany*. 61(2):145–151.

Ford M.A., Thorne G.N. 1974 Effects of Atmospheric Humidity on Plant Growth. *Annals of Botany*. 38:441–452.

Fox, J. & Weisberg, S. 2011. *An {R} Companion to Applied Regression, Second Edition*. Thousand Oaks CA: Sage.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A. 2008. Transformation of the Nitrogen Cycle : *Science*. 320:889–892.

Gerten, D., Schaphoff, S., Haberlandt, U., Lucht, W. & Sitch, S. 2004. Terrestrial vegetation and water balance - Hydrological evaluation of a dynamic global vegetation model. *Journal of Hydrology*. 286(1–4):249–270.

Ghannoum, O., Caemmerer, S. Von, Ziska, L.H. & Conroy, J.P. 2000. The growth response of C_4 plants to rising atmospheric CO_2 partial pressure: a reassessment. *Plant, Cell*

1937 *and Environment*. 23(9):931–942.

1938 Gholipoor, M., Prasad, P.V.V., Mutava, R.N. & Sinclair, T.R. 2010. Genetic variability of
1939 transpiration response to vapor pressure deficit among sorghum genotypes. *Field*
1940 *Crops Research*. 119(1):85–90.

1941 Gifford, R.M., Barrett, D.J. & Lutze, J.L. 2000. The effects of elevated [CO_2] on the C:N and
1942 C:P mass ratios of plant tissues. *Plant and Soil*. 224:1–14.

1943 Gillespie, A.R. 1989. Modelling nutrient flux and interspecies root competition in
1944 agroforestry interplantings. *Agroforestry Systems*. 8(3):257–265.

1945 Giorgi, F., Whetton, P.H., Jones, R.G., Christensen, J.H., Mearns, L.O., Hewitson, B., Von
1946 Storch, H., Francisco, R. & Jack, C. 2001. Emerging patterns of simulated regional
1947 climatic changes for the 21st century due to anthropogenic forcings. *Geophysical*
1948 *Research Letters*. 28. 3317-3320.

1949 Gisleröd, H.R. & Nelson, P. V. 1989. Effects of Humidity on Photosynthesis. *Scientia*
1950 *Horticulturae*. 38:305–313.

1951 Gojon, A., Krouk, G., Perrine-Walker, F. & Laugier, E. 2011. Nitrate transceptor(s) in plants.
1952 *Journal of Experimental Botany*. 62(7):2299–2308.

1953 De Groot, C.C., Marcelis, L.F.M., Van Den Boogaard, R., Kaiser, W.M. & Lambers, H.
1954 2003. Interaction of nitrogen and phosphorus nutrition in determining growth. *Plant*
1955 *and Soil*. 248(1–2):257–268.

1956 Hewitt, E. 1966. *Sand and water culture methods used in the study of plant nutrition*. 2nd ed.
1957 Farnham Royal, England: Common Agricultural Bureau.

1958 Hocking, P.J. & Meyer, C.P. 1991. Carbon dioxide enrichment decreases critical nitrate and
1959 nitrogen concentrations in wheat. *Journal of Plant Nutrition*. 14(6):571–584.

1960 Hoffman, G.J. 1979. Humidity. In *Controlled environment guidelines for plant research*.
1961 T.W. Tibbits & T.T. Kozlowski, Eds. London: Academic Press.

1962 Houshmandfar, A., Fitzgerald, G.J., O'Leary, G., Tausz-Posch, S., Fletcher, A. & Tausz, M.
1963 2018. The relationship between transpiration and nutrient uptake in wheat changes
1964 under elevated atmospheric CO_2. *Physiologia Plantarum*. 163(4): 516–529.

1965 Hunsaker, D.J., Kimball, B.A., Pinter, P.J., Wall, G.W., Lamorte, R.L., Adamsen, F.J.,
1966 Leavitt, S.W., Thompson, T.L., Matthias, A.D., Brooks, T.J. 2000. CO_2 enrichment
1967 and soil nitrogen effects on wheat evapotranspiration and water use efficiency.
1968 *Agricultural and Forest Meteorology*. 104(2):85–105.

1969 IPCC. 2014. *Climate change 2014: synthesis report.' Contribution of Working Groups I, II*
1970 *and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate*
1971 *Change. Core Writing Team, RK Pachauri, LA Meyer Eds.* IPCC, Geneva,
1972 Switzerland, 151

1973 Jablonski, L.M., Wang, X. & Curtis, P.S. 2002. Plant reproduction under elevated CO_2
1974 conditions: A meta-analysis of reports on 79 crop and wild species. *New Phytologist*.
1975 156(1):9–26.

1976 Jones, R.J. & Mansfield, T.A. 1970. Suppression of stomatal opening in leaves treated with
1977 abscisic acid. *Journal of Experimental Botany*. 21(3):714–719.

1978 Jyostna Devi, M., Sinclair, T.R. & Vadez, V. 2010. Genotypic variation in peanut for
1979 transpiration response to vapor pressure deficit. *Crop Science*. 50(1):191–196.

1980 Kawamitsu, Y., Yoda, S. & Agata, W. 1993. Humidity Pretreatment Affects the Responses of
1981 Stomata and CO_2 Assimilation to Vapor Pressure Difference in C_3 and C_4 Plants.
1982 *Plant Cell*. 34(1):113–119.

1983 Kimball, B.A. 2006. The Effects of Free-Air [CO_2] Enrichment of Cotton, Wheat, and
1984 Sorghum. *Managed Ecosystems and CO2*. 187:47–70.

1985 Kimball, B.A. 2016. Crop responses to elevated CO_2 and interactions with H_2O, N, and
1986 temperature. *Current Opinion in Plant Biology*. 31:36–43.

1987 Kimball, B.A. 2006. The effects of free-air [CO_2] enrichment of cotton, wheat and sorghum.
1988 In *Managed Ecosystems and CO_2. Case Studies, Processes and Perspectives*. S.P.J.
1989 Nösberger, S.P. Long, R.J. Norby, M. Stitt, Eds. Heidelberg, Berlin: Springer-Verlag.
1990 47–70.

1991 Kirkham, M.B. 2005. Field Capacity, Wilting Point, Available Water, and the Non-Limiting
1992 Water Rang. In *Principles of Soil and Plant Water Relations*. M.B. Kirkham, Ed.
1993 Academic Press. 101–115.

1994 Kling, G.W., Hayhoe, K., Johnson, L.B., Magnuson, J.J., Polasky, S., Robinson, S.K., Shuter,
1995 B.J., Wander, M.M., Wubbles, D., Donald, Z. 2003. *Confronting Climate Change in*
1996 *the Great Lakes Region: Impacts on our Communities and Ecosystems.* Union of
1997 Concerned Scientists, Cambridge, Massachusetts, and Ecological Society of America,
1998 Washington, D.C.

1999 Körner, C. 2006. Plant CO_2 responses: An issue of definition, time and resource supply. *New*
2000 *Phytologist*. 172(3):393–411.

2001 Kramer, P.J. & Boyer, J.N. 1995. *Water Relations of Plants and Soils*. San Diego: Academic
2002 Press.

2003 Kuehny, J.S., Peet, M.M., Nelson, P. V. & Willits, D.H. 1991. Nutrient Dilution by Starch in
2004 CO_2-enriched *Chrysanthemum*. *Journal of Experimental Botany*. 42(6):711–716.

2005 Kuwagata, T., Ishikawa-Sakurai, J., Hayashi, H., Nagasuga, K., Fukushi, K., Ahamed, A.,

Takasugi, K., Katsuhara, M., Murai-Hatano, M. 2012. Influence of low air humidity and low root temperature on water uptake, growth and aquaporin expression in rice plants. *Plant and Cell Physiology*. 53(8):1418–1431.

Lambers, H., Chapin, F.I. & Pons, T. 1998. *Plant physiological ecology*. New York, NY, USA: Springer-Verlag New York.

Lange, O.L., Schulze, E.-D., Kappen, L., Buschbom, U. & Evenari, M. 1975. Photosynthesis of desert plants as influenced by internal and external factors. In *Perspectives in Biophysical Ecology. Ecological Studies v. 12*. D.M. Gates & R.D. Schmerl, Eds. Springer-Verlag, New York. 121–143.

Larcher, W. 1995. *Physiological plant ecology*. Springer-Verlag, New York.

Leakey, A.D.B. 2009. Rising atmospheric carbon dioxide concentration and the future of C_4 crops for food and fuel. *Proceedings of the Royal Society*. 276(1666):2333–43.

Leakey, A.D.B., Bernacchi, C.J., Dohleman, F.G., Ort, D.R. & Long, S.P. 2004. Will photosynthesis of maize (*Zea mays*) in the US Corn Belt increase in future [CO_2] rich atmospheres? An analysis of diurnal courses of CO_2 uptake under free-air concentration enrichment (FACE). *Global Change Biology*. 10(6):951–962.

Leakey, A.D.B., Uribelarreà, M., Ainsworth, E.A., Naidu, S.L., Rogers, A., Ort, D.R. & Long, S.P. 2006. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. *Plant Physiology*. 140(2):779–790.

Leakey, A.D.B., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P. & Ort, D.R. 2009. Elevated CO2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *Journal of Experimental Botany*. 60(10):2859–2876.

2029 Russell Lenth (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means. R
2030 package version 1.4.1. https://CRAN.R-project.org/package=emmeans

2031 Leuschner, C. 2002. Air humidity as an ecological factor for woodland herbs: Leaf water
2032 status, nutrient uptake, leaf anatomy, and productivity of eight species grown at low
2033 or high vpd levels. *Flora*. 197(4):262–274. DOI: 10.1078/0367-2530-00040.

2034 Li, F., Kang, S. & Zhang, J. 2004. Interactive effects of elevated CO_2, nitrogen and drought
2035 on leaf area, stomatal conductance, and evapotranspiration of wheat. *Agricultural*
2036 *Water Management*. 67(3):221–233.

2037 Lieffering, M., Kim, H.Y., Kobayashi, K. & Okada, M. 2004. The impact of elevated CO_2 on
2038 the elemental concentrations of field-grown rice grains. *Field Crops Research*. 88(2–
2039 3):279–286.

2040 Lihavainen, J., Ahonen, V., Keski-Saari, S., Kontunen-Soppela, S., Oksanen, E. & Keinänen,
2041 M. 2016. Low vapour pressure deficit affects nitrogen nutrition and foliar metabolites
2042 in silver birch. *Journal of Experimental Botany*. 67(14):4353–4365.

2043 Lobell, D.B., Roberts, M.J., Schlenker, W., Braun, N., Little, B.B., Rejesus, R.M. &
2044 Hammer, G.L. 2014. Greater sensitivity to drought accompanies maize yield increase
2045 in the U.S. Midwest. *Science*. 344(6183):516–519.

2046 Loladze, I. 2002. Rising atmospheric CO2 and human nutrition: Toward globally imbalanced
2047 plant stoichiometry? *Trends in Ecology and Evolution*. 17(10):457–461.

2048 Loladze, I. 2014. Hidden shift of the ionome of plants exposed to elevated CO_2 depletes
2049 minerals at the base of human nutrition. *eLife*. 2014(3):1–29.

2050 Long, S.P., Ainsworth, E.A., Rogers, A. & Ort, D.R. 2004. RISING ATMOSPHERIC
2051 CARBON DIOXIDE: Plants FACE the Future. *Annual Review of Plant Biology*.

55(1):591–628.

Luo, J., Li, H., Liu, T., Polle, A., Peng, C. & Luo, Z. Bin. 2013. Nitrogen metabolism of two contrasting poplar species during acclimation to limiting nitrogen availability. *Journal of Experimental Botany*. 64(14):4207–4224.

Madhavan, S., Treichel, I. & O'Leary, M.H. 1991. Effects of Relative Humidity on Carbon Isotope Fractionation in Plants. *Botanica Acta*. 104(4):292–294.

Mae, T. 1997. Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. *Plant and Soil*. 196:201-210

Makino, A. 2003. Rubisco and nitrogen relationships in rice: Leaf photosynthesis and plant growth. *Soil Science and Plant Nutrition*. 49(3):319–327.

Manderscheid, R., Erbs, M. & Weigel, H.J. 2014. Interactive effects of free-air CO_2 enrichment and drought stress on maize growth. *European Journal of Agronomy*. 52:11–21.

Markelz, R.J.C., Strellner, R.S. & Leakey, A.D.B. 2011. Impairment of C_4 photosynthesis by drought is exacerbated by limiting nitrogen and ameliorated by elevated [CO_2] in maize. *Journal of Experimental Botany*. 62(9):3235–3246.

Marschner, H. 1995. *Mineral nutrition of higher plants*. *San Diego*. Academic Press.

Marschner, H. & Marschner, P. 2012. *Marschner's mineral nutrition of higher plants*. London: Academic Press.

Marschner, P. & Rengel, Z. 2012. Nutrient Availability in Soils. *Marschner's mineral nutrition of higher plants*. 315-330.

McDonald, E.P., Erickson, J.E. & Kruger, E.L. 2002. Can decreased transpiration limit plant

nitrogen acquisition in elevated CO_2? *Functional Plant Biology*. 29(9):1115–1120.

Mcgrath, J.M. & Lobell, D.B. 2013. Reduction of transpiration and altered nutrient allocation contribute to nutrient decline of crops grown in elevated CO_2 concentrations. *Plant, Cell and Environment*. 36(3):697–705.

McMurtrie, R.E., Norby, R.J., Medlyn, B.E., Dewar, R.C., Pepper, D.A., Reich, P.B. & Barton, C.V.M. 2008. Why is plant-growth response to elevated CO_2 amplified when water is limiting, but reduced when nitrogen is limiting? A growth-optimisation hypothesis. *Functional Plant Biology*. 35(6):521–534.

Moore, P.H. & Botha, F.C. 2014. *Sugarcane Physiology Biochemistry & Functional Biology*.

Morison, J.I.L. & Gifford, R.M. 1983. Stomatal Sensitivity to Carbon Dioxide and Humidity. *Plant Physiology*. 71(4):789–796.

Mortensen, L.M. 1987. Review: CO_2 enrichment in greenhouses. Crop Responses. *Scientia Horticulturae*. 33(1–2):1–25.

Mott, K.A. & Parkhurst, D.F. 1991. Stomatal responses to humidity in air and helox. *Plant, Cell & Environment*. 14(5):509–515.

Mu, Q., Zhao, M., Heinsch, F.A., Liu, M., Tian, H. & Running, S.W. 2007. Evaluating water stress controls on primary production in biogeochemical and remote sensing based models. *Journal of Geophysical Research: Biogeosciences*. 112(1):1–13.

Myers, S.S., Zanobetti, A., Kloog, I., Huybers, P., Leakey, A.D.B., Bloom, A.J., Carlisle, E., Dietterich, L.H., Fitzgerald, G., Toshihiro, H., Holbrook, N. M., Nelson, R.L., Ottman, M.J., Raboy, V., Sakai, H., Sartor, K.A., Schwartz, J., Seneweera, S., Tausz, M., Usui, Y.. 2014. Increasing CO_2 threatens human nutrition. *Nature*. 510(7503):139–42.

2097 Ntanos, D.A. & Koutroubas, S.D. 2002. Dry matter and N accumulation and translocation for
2098 Indica and Japonica rice under Mediterranean conditions. *Field Crops Research*.
2099 74(1):93–101.

2100 Oliveira, E.M.M., Ruiz, H.A., Alvarez V, V.H., Ferreira, P.A., Costa, F.O. & Almeida, I.C.C.
2101 2010. Nutrient supply by mass flow and diffusion to maize plants in response to soil
2102 aggregate size and water potential. *Revista Brasileira de Ciência do Solo*. 34(2):317–
2103 328.

2104 Oren, R., Sperry, J.S., Katul, G.G., Pataki, D.E., Ewers, B.E., Phillips, N. & Schäfer, K.V.R.
2105 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity
2106 to vapour pressure deficit. *Plant, Cell and Environment*. 22(12):1515–1526.

2107 Oyewole, O.A., Inselsbacher, E. & Näsholm, T. 2014. Direct estimation of mass flow and
2108 diffusion of nitrogen compounds in solution and soil. *New Phytologist*. 201(3):1056–
2109 1064.

2110 Papakosta, D.K. & Gagianas, A.A. 1991. Nitrogen and Dry Matter Accumulation,
2111 Remobilization, and Losses for Mediterranean Wheat during Grain Filling. *Agronomy*
2112 *Journal*. 83(5):864.

2113 Parts, K., Tedersoo, L., Lõhmus, K., Kupper, P., Rosenvald, K., Sõber, A. & Ostonen, I.
2114 2013. Increased air humidity and understory composition shape short root traits and
2115 the colonizing ectomycorrhizal fungal community in silver birch stands. *Forest*
2116 *Ecology and Management*. 310:720–728.

2117 Paterson, E., Sim, A., Standing, D., Dorward, M. & McDonald, A.J.S. 2006. Root exudation
2118 from Hordeum vulgare in response to localized nitrate supply. *Journal of*
2119 *Experimental Botany*. 57(10):2413–2420.

2120 Peirson, D.R. & Elliott, J.R. 1988. Effect of Nitrite and Bicarbonate on Nitrite Utilization in
2121 Leaf Tissue of Bush Bean (*Phaseolus vulgaris*). *Journal of Plant Physiology*.
2122 133(4):425–429.

2123 Pleijel, H., Mortensen, L., Fuhrer, J., Ojanperä, K. & Danielsson, H. 1999. Grain protein
2124 accumulation in relation to grain yield of spring wheat (*Triticum aestivum* L.) grown
2125 in open-top chambers with different concentrations of ozone, carbon dioxide and
2126 water availability. *Agriculture, Ecosystems and Environment*. 72(3):265–270.

2127 Pleijel, H., Broberg, M.C., Högy, P. & Uddling, J. 2019. Nitrogen application is required to
2128 realize wheat yield stimulation by elevated CO 2 but will not remove the CO_2-induced
2129 reduction in grain protein concentration. *Global Change Biology*. 25(5):1868–1876.

2130 Poorter, H., Van Berkel, Y., Baxter, R., Den Hertog, J., Dijkstra, P., Gifford, R.M., Griffin,
2131 K.L., Roumet, C., Wong, S.C. 1997. The effect of elevated CO_2 on the chemical
2132 composition and construction costs of leaves of 27 C_3 species. *Plant, Cell and*
2133 *Environment*. 20(4):472–482.

2134 Porteaus, F., Hill, J., Ball, A.S., Pinter, P.J., Kimball, B.A., Wall, G.W., Adamsen, F.J.,
2135 Hunsaker, D.J., LaMorte, R.L., Leavitt, S.W., Thompson, T.L., Matthias, A.D.,
2136 Brooks, T.J., Morris, C.F. 2009. Effect of Free Air Carbon dioxide Enrichment
2137 (FACE) on the chemical composition and nutritive value of wheat grain and straw.
2138 *Animal Feed Science and Technology*. 149(3–4):322–332.

2139 del Pozo, A., Perez, P., Gutierrez, D., Alonso, A., Morcuende, R. & Martinez-Carrasco, R.
2140 2007. Gas exchange acclimation to elevated CO_2 in upper-sunlit and lower-shaded
2141 canopy leaves in relation to nitrogen acquisition and partitioning in wheat grown in
2142 field chambers. *Environmental and Experimental Botany*. 59:371–380.

2143 Pritchard, S. & Rogers, H. 2000. Spatial and temporal deployment of crop. *New Phytologist*.

147:55–71.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ranathunge, K., Steudle, E. & Lafitte, R. 2003. Control of water uptake by rice (*Oryza sativa* L.): role of the outer part of the root. *Planta*. 217:193–205.

Rathnam, C.K.M. 1978. Malate and Dihydroxyacetone Phosphate-dependent Nitrate Reduction in Spinach Leaf Protoplasts. *Plant Physiology*. 62(2):220–223.

Reeves, D.W., Rogers, H.H., Prior, S.A., Wood, C.W. & Runion, G.B. 1994. Elevated atmospheric carbon dioxide effects on sorghum and soybean nutrient status. *Journal of Plant Nutrition*. 17(11):1939–1954.

Reich, P.B., Walters, M.B. & Ellsworth, D.S. 1997. From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences*. 94:13730–13734.

Robinson, S.P. & Giersch, C. 1987. Inorganic Phosphate Concentration in the Stroma of Isolated Chloroplasts and Its Influence on Photosynthesis. *Australian Journal of Plant Physiology*. 14(4):451-462

Rogers, H.H. & Runion, G.B. 1994. Plant reponses to atmospheric CO2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution*. 83:155–189.

Rose, L., Buitenwerf, R., Cramer, M., February, E.C. & Higgins, S.I. 2018. Effects of nutrient supply on carbon and water economies of C4 grasses. *Functional Plant Biology*. 45(9):935–944.

Rosenvald, K., Tullus, A., Ostonen, I., Uri, V., Kupper, P., Aosaar, J., Varik, M., Sõber, J., Niglas, A., Hansen, R., Rohula, G., Kukk, M., Sober, A., Lohmus, K. 2014. The effect

of elevated air humidity on young silver birch and hybrid aspen biomass allocation and accumulation - acclimation mechanisms and capacity. *Forest Ecology and Management*. 330:252–260.

Rufty, T.W., Thomas, J.F., Remmler, J.L., Campbell, W.H. & Volk, R.J. 1986. Intercellular Localization of Nitrate Reductase in Roots. *Plant Physiology*. 82(3):675–680.

Russell, R.S. & Barber, S.A. 1960. The Relationship Between Salt Uptake and the Absorption of Water by Intact Plants. *Annual Review of Plant Physiology*. 11:127–140.

Sage, R.F. 1994. Acclimation of photosynthesis to increasing atmospheric CO_2: The gas exchange perspective. *Photosynthesis Research*. 39(3):351–368.

Sanchez-Diaz, M., Garcia, J.L., Antolin, M.C. & Araus, J.L. 2002. Effects of soil drought and atmospheric humidity on yield, gas exchange, and stable carbon isotope composition of barley. *Photosynthetica*. 40(3):415–421.

Schmidt, I.K., Tietema, A., Williams, D., Gundersen, P., Beier, C., Emmett, B.A. & Estiarte, M. 2004. Soil Solution Chemistry and Element Fluxes in Three European Heathlands and Their Responses to Warming and Drought. *Ecosystems*. 7(6):638–649.

Searles, P.S. & Bloom, A.J. 2003. Nitrate photo-assimilation in tomato leaves under short-term exposure to elevated carbon dioxide and low oxygen. 1247–1255.

Sellin, A., Tullus, A., Niglas, A., Õunapuu, E., Karusion, A. & Lõhmus, K. 2013. Humidity-driven changes in growth rate, photosynthetic capacity, hydraulic properties and other functional traits in silver birch (*Betula pendula*). *Ecological Research*. 28(3):523–535.

Sellin, A., Rosenvald, K., Õunapuu-Pikas, E., Tullus, A., Ostonen, I. & Lõhmus, K. 2015. Elevated air humidity affects hydraulic traits and tree size but not biomass allocation

in young silver birches (*Betula pendula*). *Frontiers in Plant Science*. 6:1–11.

Sharkey, T.D. 1984. Transpiration-induced changes in the photosynthetic capacity of leaves. *Planta*. 160(2):143–150. DOI: 10.1007/BF00392862.

Simpson, R.J., Lambers, H. & Dalling, M.J. 1983. Nitrogen Redistribution during Grain Growth in Wheat (*Triticum aestivum* L.) . *Plant Physiology*. 71(1):7–14.

Sinclair, T.R., Zwieniecki, M.A. & Holbrook, N.M. 2008. Low leaf hydraulic conductance associated with drought tolerance in soybean. *Physiologia Plantarum*. 132(4):446–451.

Singh, S. 2016. Guttation: Mechanism, Momentum and Modulation. *Botanical Review*. 82(2):149–182.

Slewinski, T.L., Meeley, R. & Braun, D.M. 2009. Sucrose transporter1 functions in phloem loading in maize leaves. *Journal of Experimental Botany*. 60(3):881–892.

Smith, M.R. & Myers, S.S. 2019. Global Health Implications of Nutrient Changes in Rice Under High Atmospheric Carbon Dioxide. *GeoHealth*. 3(7):190–200 .

Smith, P., Davis, S.J., Creutzig, F., Fuss, S., Minx, J., Gabrielle, B., Kato, E., Jackson, R.B., et al. 2016. Biophysical and economic limits to negative CO_2 emissions. *Nature Climate Change*. 6(1):42–50.

Stimler, K., Berry, J.A. & Yakir, D. 2012. Effects of carbonyl sulfide and carbonic anhydrase on stomatal conductance. *Plant Physiology*. 158(1):524–530.

Stitt, M. & Krapp, A. 1999. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell and Environment*. 22:553–621.

2212 Taiz, L. & Zeiger, E. 2010. *Plant Physiology (Fifth Edition)*. V. 1.

2213 Tanner, W. & Beevers, H. 2001. Transpiration, a prerequisite for long-distance transport of
2214 minerals in plants? *Proceedings of the National Academy of Sciences of the United*
2215 *States of America*. 98(16):9443–9447.

2216 Tanner, W. & Beevers, H. 1990. Does transpiration have an essential function in long-
2217 distance ion transport in plants? *Plant, Cell & Environment*. 13(8):745–750.

2218 Taub, D.R. & Wang, X. 2008. Why are nitrogen concentrations in plant tissues lower under
2219 elevated CO_2? A critical examination of the hypotheses. *Journal of Integrative Plant*
2220 *Biology*. 50(11):1365–1374.

2221 Taub, D.R., Miller, B. & Allen, H. 2008. Effects of elevated CO_2 on the protein concentration
2222 of food crops: a meta-analysis. *Global Change Biology*. 14(3):565–575. DOI:
2223 10.1111/j.1365-2486.2007.01511.x.

2224 Tullus, A., Kupper, P., Sellin, A., Parts, L., Sõber, J., Tullus, T., Lõhmus, K., Sõber, A.,
2225 Tullus, H. 2012. Climate change at Northern latitudes: Rising atmospheric humidity
2226 decreases transpiration, N-uptake and growth rate of hybrid aspen. *PLoS ONE*. 7(8):
2227 e42648

2228 Turner, N.C., Schulze, E. & Gollan, T. 1985. The Responses of Stomata and Leaf Gas
2229 Exchange to Vapour Pressure Deficits and Soil Water Content . II. In the Mesophytic
2230 Herbaceous Species *Helianthus annuus*. *Oecologia*. 65(3):348–355.

2231 Uddling, J., Broberg, M.C., Feng, Z. & Pleijel, H. 2018. Crop quality under rising
2232 atmospheric CO_2. *Current Opinion in Plant Biology*. 45:262–267.

2233 Vandamme, E., Renkens, M., Pypers, P., Smolders, E., Vanlauwe, B. & Merckx, R. 2013.
2234 Root hairs explain P uptake efficiency of soybean genotypes grown in a P-deficient

Ferralsol. *Plant and Soil*. 369(1–2):269–282.

Van Vuuren, M.M.I., Robinson, D., Fitter, A.H., Chasalow, S.D., Williamson, L. & Raven, J.A. 1997. Effects of elevated atmospheric CO_2 and soil water availability on root biomass, root length, and N, P and K uptake by wheat. *New Phytologist*. 135(3):455–465.

Wallace, M.B. & Knausenenberger, W.I. 1997. Inorganic fertilizer use in africa : *Environmental and economic dimensions*. 10–13

Wand, S.J.E., Midgley, G.F., Jones, M.H. & Curtis, P.S. 1999. Responses of wild C_4 and C_4 grass (Poaceae) species to elevated atmospheric CO_2 concentrations: a meta-analytic test of current theories and perceptions. *Global Change Biology*. 5:723–741.

Webber, A.N., Nie, G.Y. & Long, S.P. 1994. Acclimation of photosynthetic proteins to rising atmospheric CO_2. *Photosynthesis Research*. 39(3):413–425.

Weltzin, J.F., Loik, M.E., Schwinning, S., Williams, D.G., Fay, P.A., Haddad, B.M., Harte, J., Huxman, T.E., Knapp, A.K., Lin, G., Pockman, W.T., Shaw, R.M., Small, E.E., Smith, M.D., Smith, S.D., Tissue, D.T., Zak, J.C. 2003. Assessing the Response of Terrestrial Ecosystems to Potential Changes in Precipitation. *BioScience*. 53(10):941.

Wen, Z., Li, H., Shen, J. & Rengel, Z. 2017. Maize responds to low shoot P concentration by altering root morphology rather than increasing root exudation. *Plant and Soil*. 416(1–2):377–389.

H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

Winter, K., Holtum, J.A.M., Edwards, G.E. & O'leary, M.H. 1982. Effect of low relative humidity on $\delta^{13}C$ value in two C_3 grasses and in *Panicum milioides*, a C_3-C_4 intermediate species. *Journal of Experimental Botany*. 33(1):88–91.

2258 Wong, S.C. 1979. Elevated atmospheric partial pressure of CO_2 and plant growth - I.
2259 Interactions of nitrogen nutrition and photosynthetic capacity in C_3 and C_4 plants.
2260 *Oecologia*. 44(1):68–74.

2261 Wong, S.C. 1990. Elevated atmospheric partial pressure of CO_2 and plant growth - II. Non-
2262 structural carbohydrate content in cotton plants and its effect on growth parameters.
2263 *Photosynthesis Research*. 23(2):171–180.

2264 Woodward, R.G. & Begg, J.E. 1976. The effect of atmospheric humidity on the yield of
2265 soybean. *Australian Journal of Agricultural Research*. 27(4):501–508.

2266 Wright, G.C., Rao, R.C.N. & Farquhar, G.D. 1994. Water-Use Efficiency and Carbon Isotope
2267 Discrimation in Peanut under Water Deficit Conditions. *Crop Science*. 34:92–97.

2268 Xu, G., Fan, X. & Miller, A.J. 2012. Plant Nitrogen Assimilation and Use Efficiency. *Annual*
2269 *Review of Plant Biology*. 63(1):153–182.

2270 Xue, Q., Weiss, A., Arkebauer, T.J. & Baenziger, P.S. 2004. Influence of soil water status
2271 and atmospheric vapor pressure deficit on leaf gas exchange in field-grown winter
2272 wheat. *Environmental and Experimental Botany*. 51(2):167–179.

2273 Yang, Z., Sinclair, T.R., Zhu, M., Messina, C.D., Cooper, M. & Hammer, G.L. 2012.
2274 Temperature effect on transpiration response of maize plants to vapour pressure
2275 deficit. *Environmental and Experimental Botany*. 78:157–162.

2276 Zhao, F.J. & McGrath, J.M. 2002. Assessing bioavailability of zinc, cadmium and copper in
2277 soils to the indicator plant Lepidium heterophyllum. In *COST Action 837. 4th WG2*
2278 *Workshop, Bordeaux'2002 – Risk assessment and sustainable land management using*
2279 *plants in trace element-contaminated soils.* Bordeaux.

2280 Zhu, C., Kobayashi, K., Loladze, I., Zhu, J., Jiang, Q., Xu, X., Liu, G., Seneweera, S., Ebi,

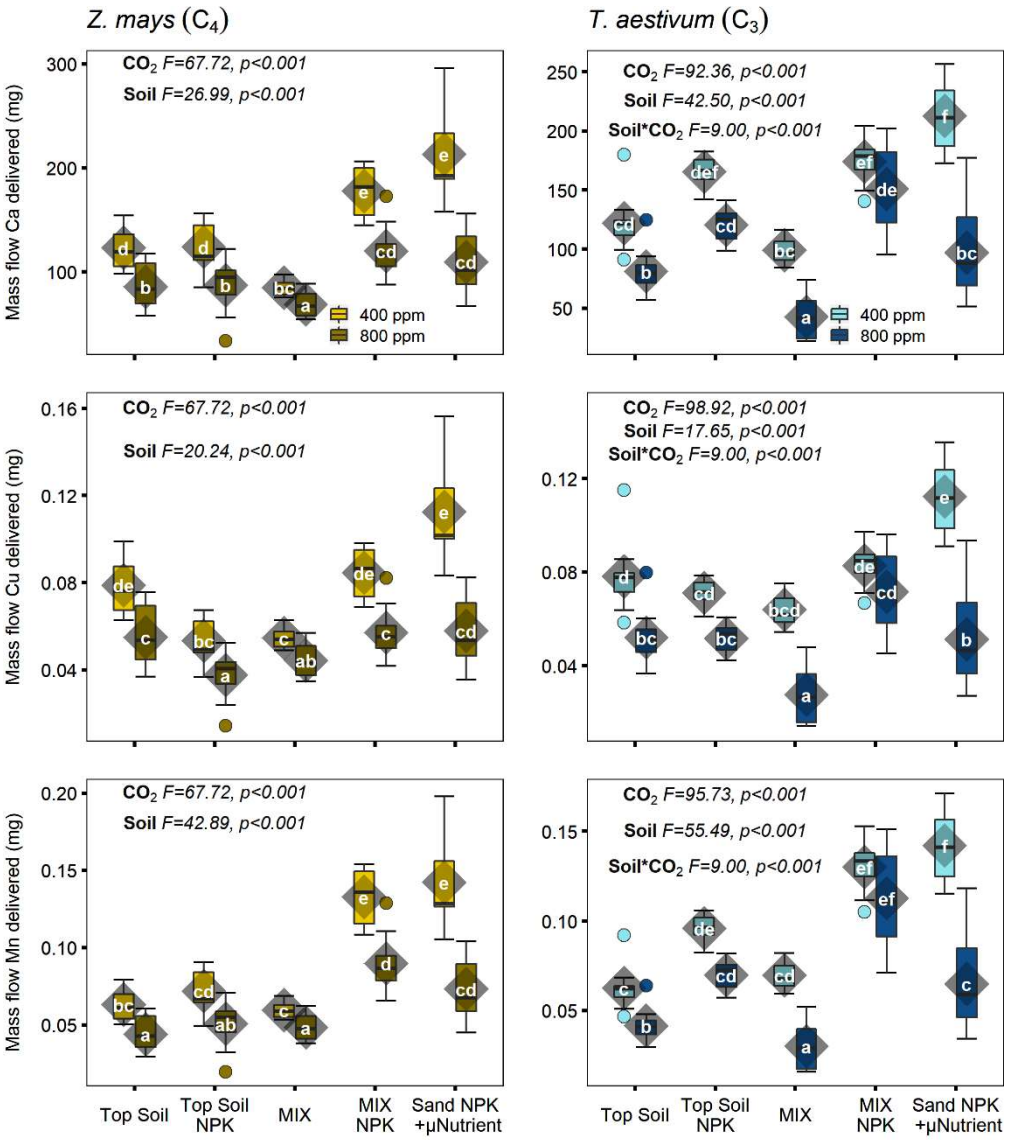
2281 K.L., Drewnowski, A., Fukagawa, N.K., Ziska, L.H. 2018. Carbon dioxide (CO_2)
2282 levels this century will alter the protein, micronutrients and vitamin content of rice
2283 grains with potential health consequences for the poorest rice-dependent countries.
2284 *Science Advances*. 4:1–9.

2285 Ziska, L.H. & Bunce, J.A. 1997. Influence of increasing carbon dioxide concentration on the
2286 photosynthetic and growth stimulation of selected C_4 crops and weeds. *Photosynthesis*
2287 *Research*. 54(3):199–208.

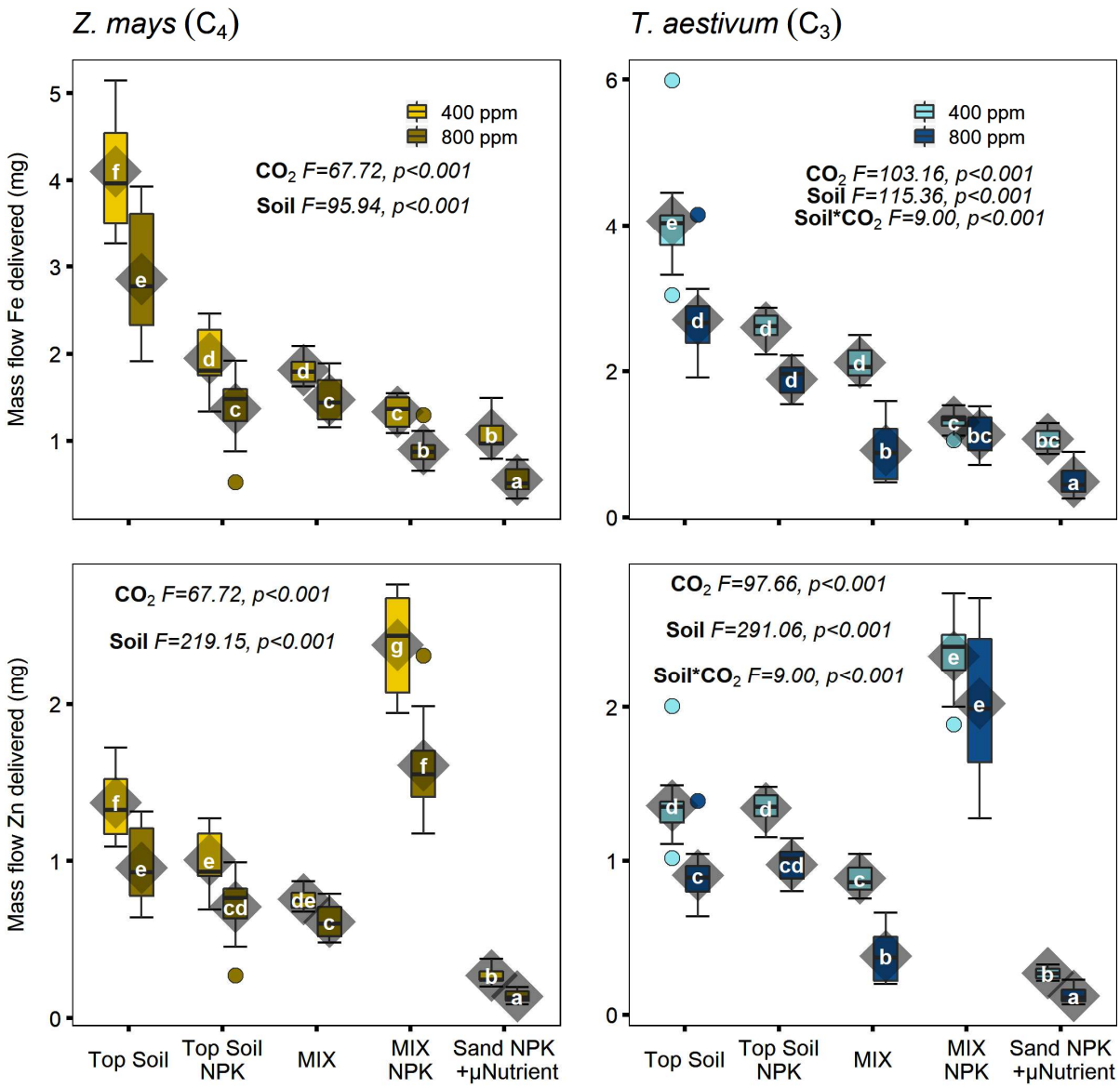
2288

2289

Appendices



Appendix 1 Variation in transpiration-driven mass flow delivery of Ca, Cu and Mn in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. The boxes and horizontal lines represent the first and the third quartiles and the medians, respectively. The whiskers represent 1.5 × the interquartile range and outliers above/below are shown as points. The diamonds represent the mean values. The F- and p-values of the main effects and interaction (when significant) from the analysis of variance done on the linear models fitted are displayed. Lower case letters indicate the significant differences between treatments as determined by Tukey's post-hoc comparisons.



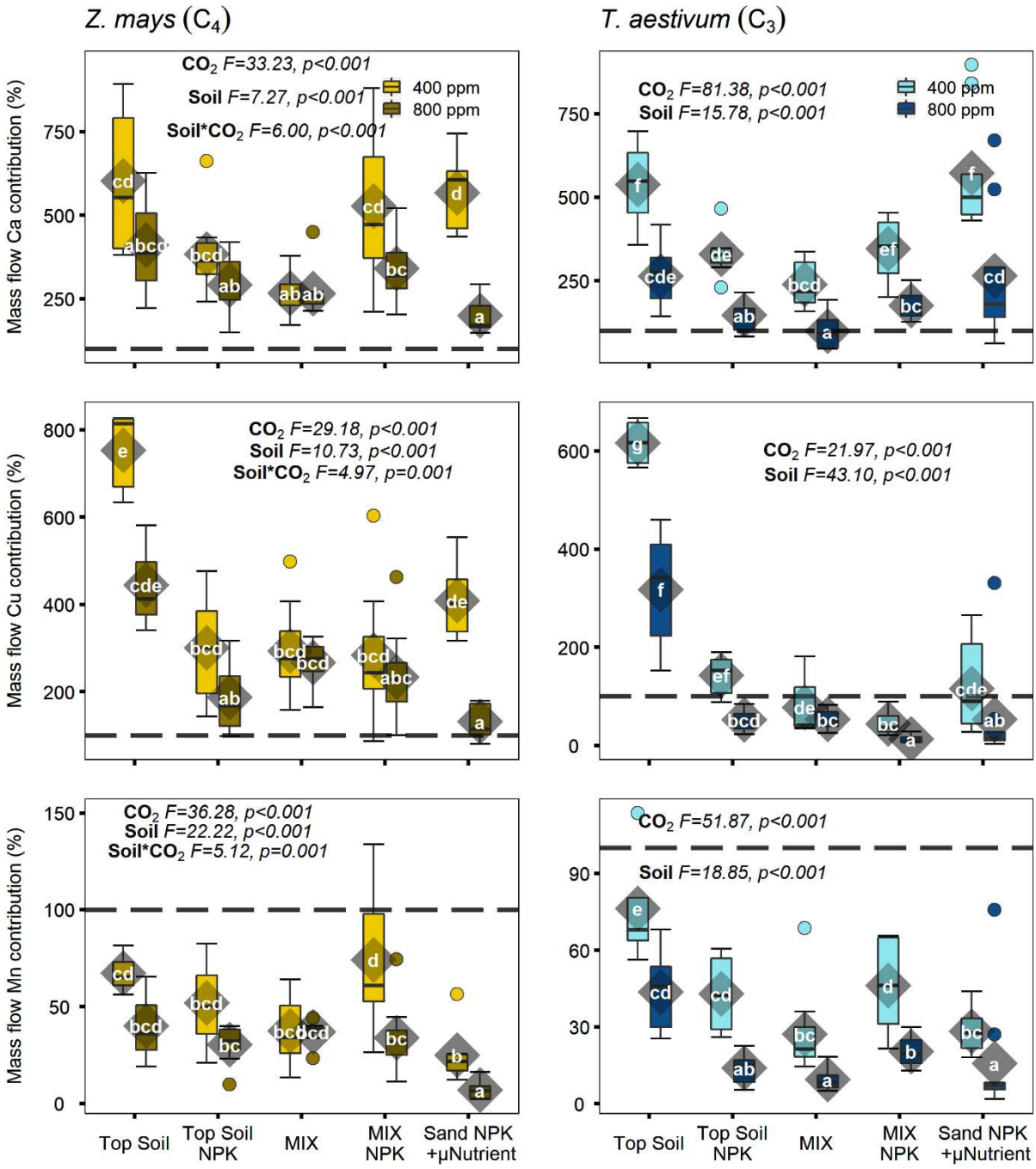
2300

2301 **Appendix 2** Variation in transpiration-driven mass flow delivery of Fe and Zn in maize and

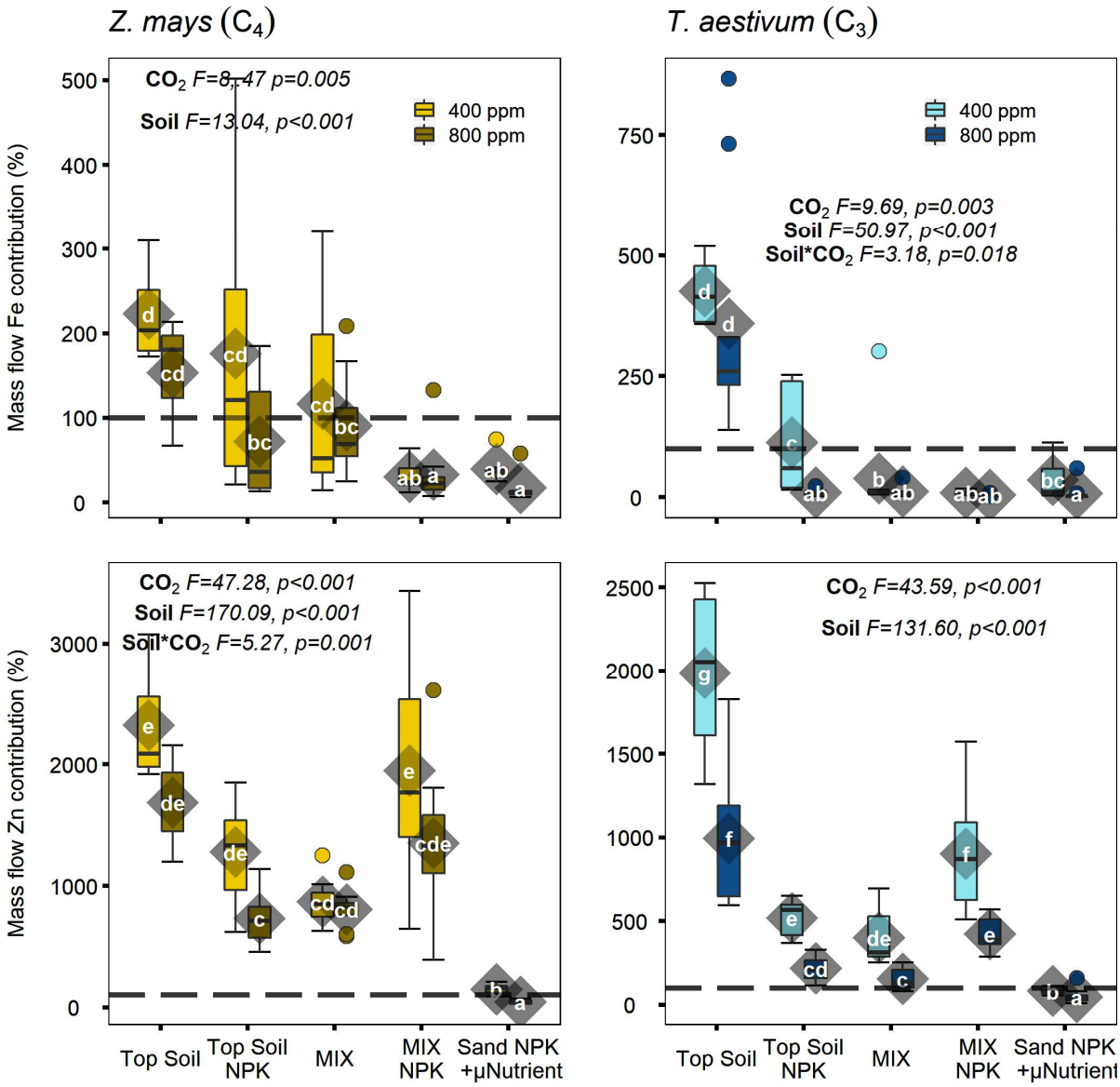
2302 wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400

2303 and 800 ppm. Statistics as in Appendix 1.

2304



Appendix 3 Variation in transpiration-driven mass flow contribution of Ca, Cu and Mn to tissue content in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. The dashed line represents 100% contribution to tissue content. Statistics as in Appendix 1.



2311

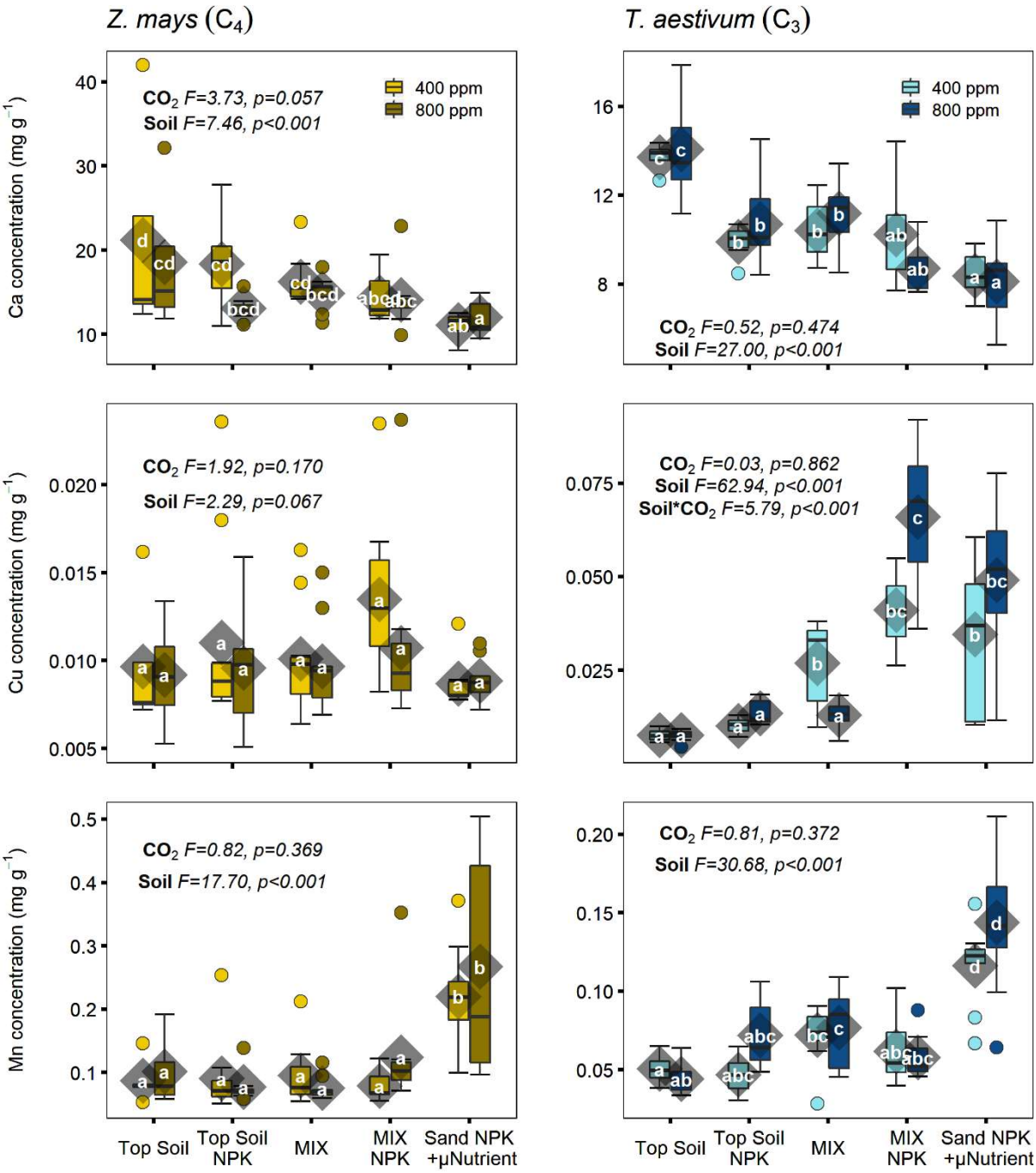
2312 **Appendix 4** Variation in transpiration-driven mass flow contribution of Fe and Zn to tissue

2313 content in maize and wheat plants, grown in five soils with differing nutrient availabilities at

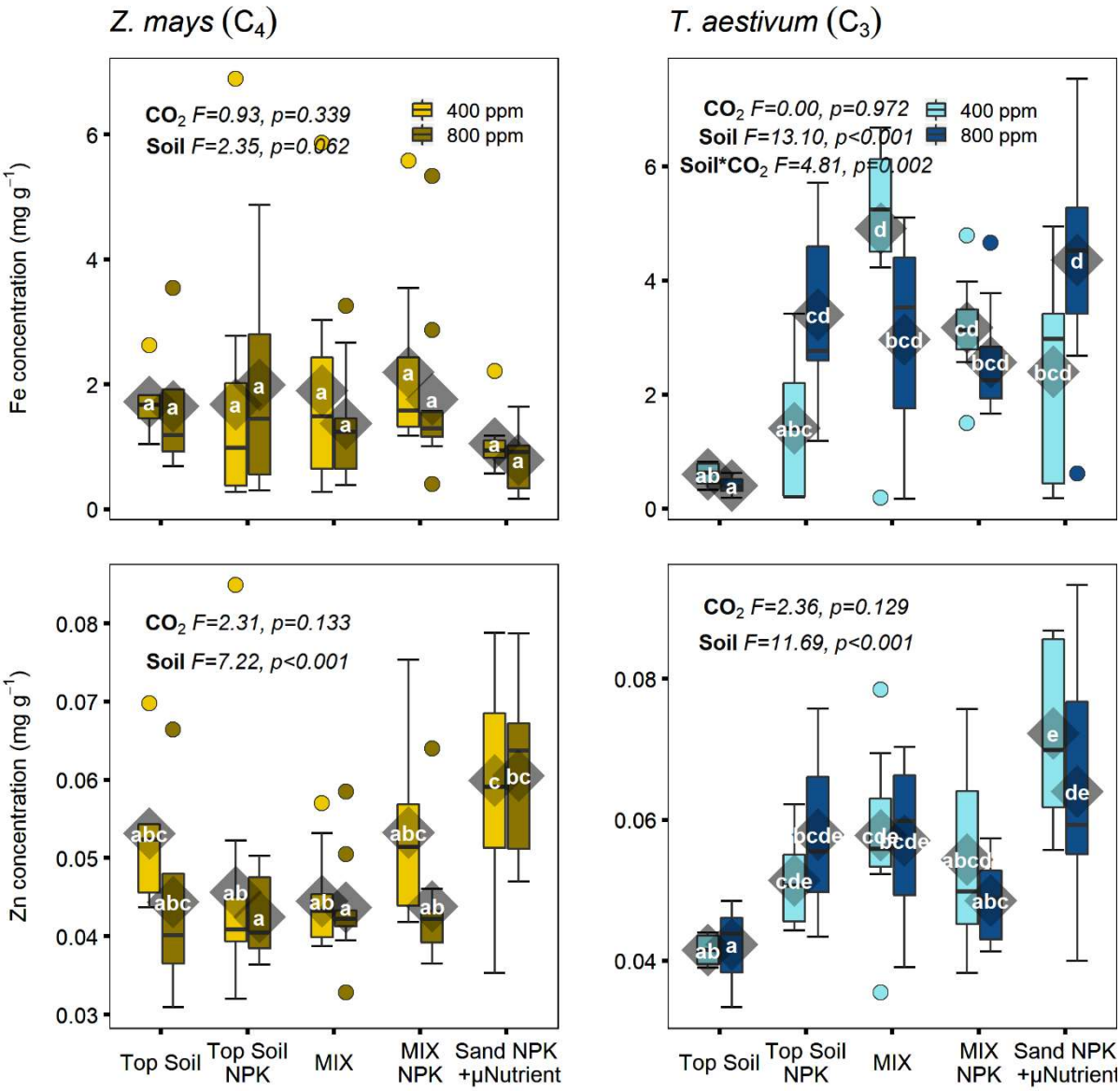
2314 two CO_2 levels, 400 and 800 ppm. The dashed line represents 100% contribution to tissue

2315 content. Statistics as in Appendix 1.

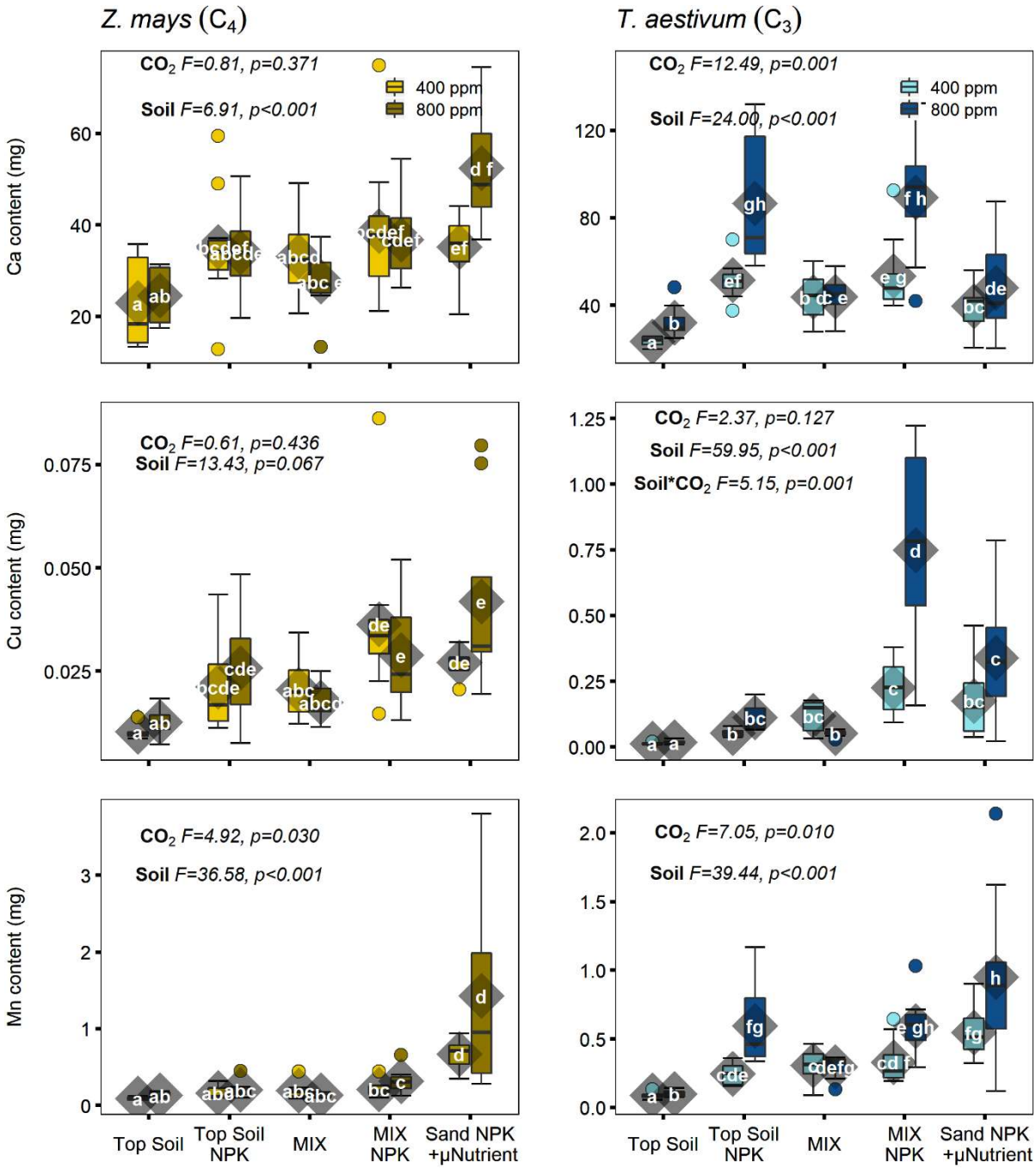
2316



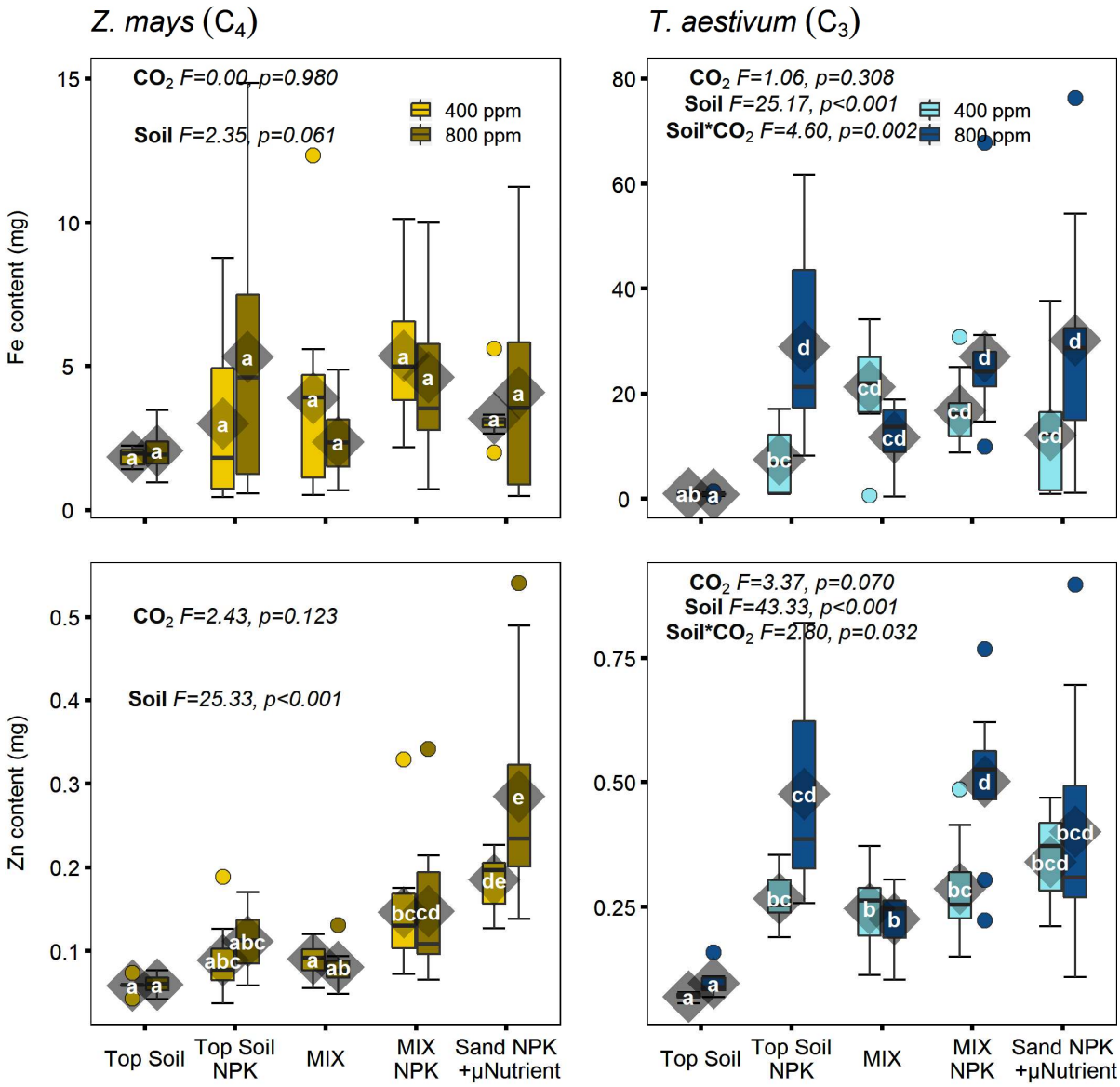
Appendix 5 Variation in total tissue concentrations of Ca, Cu and Mn in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Appendix 1.



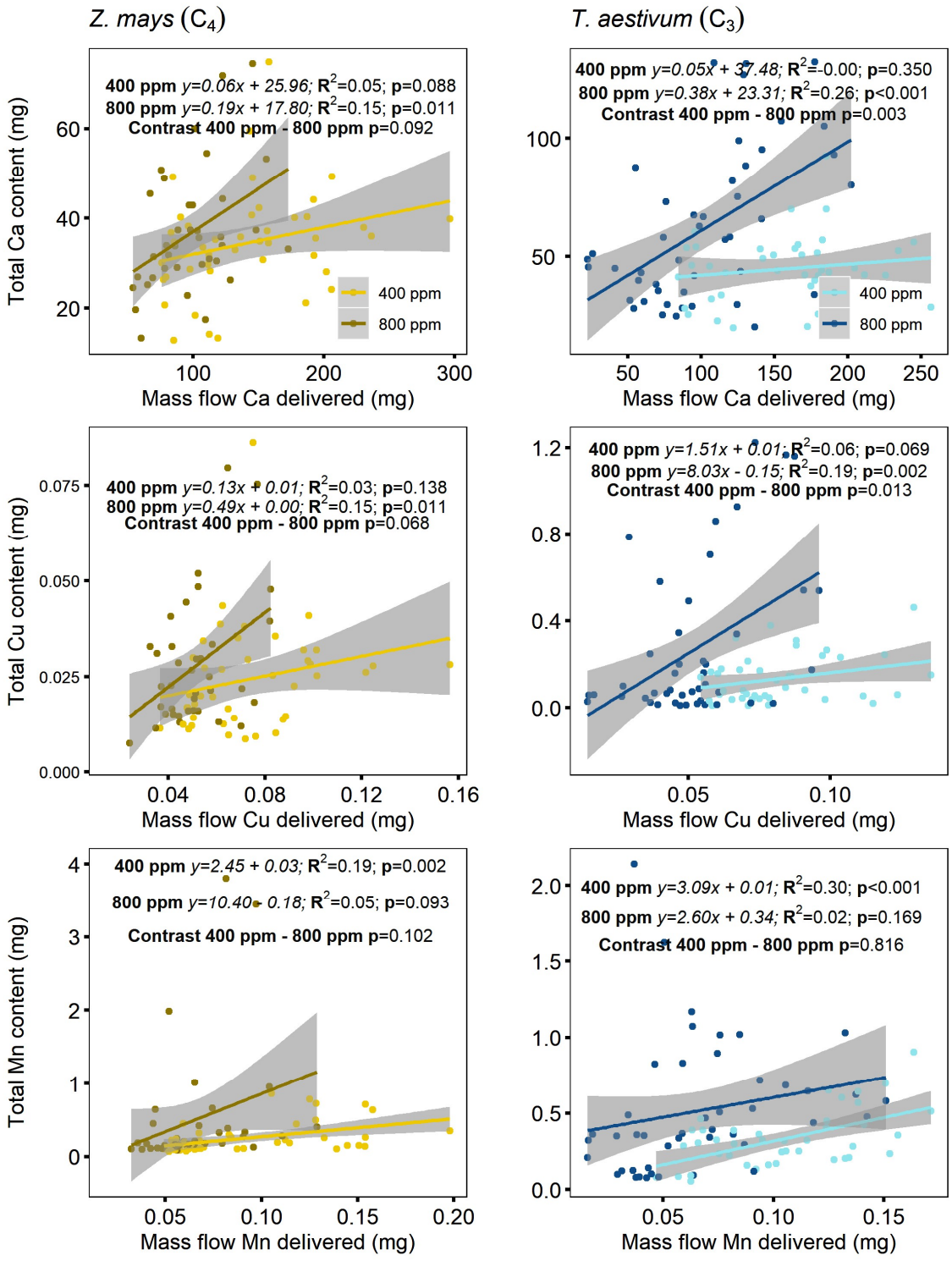
Appendix 6 Variation in total tissue concentrations of Fe and Zn in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Appendix 1.



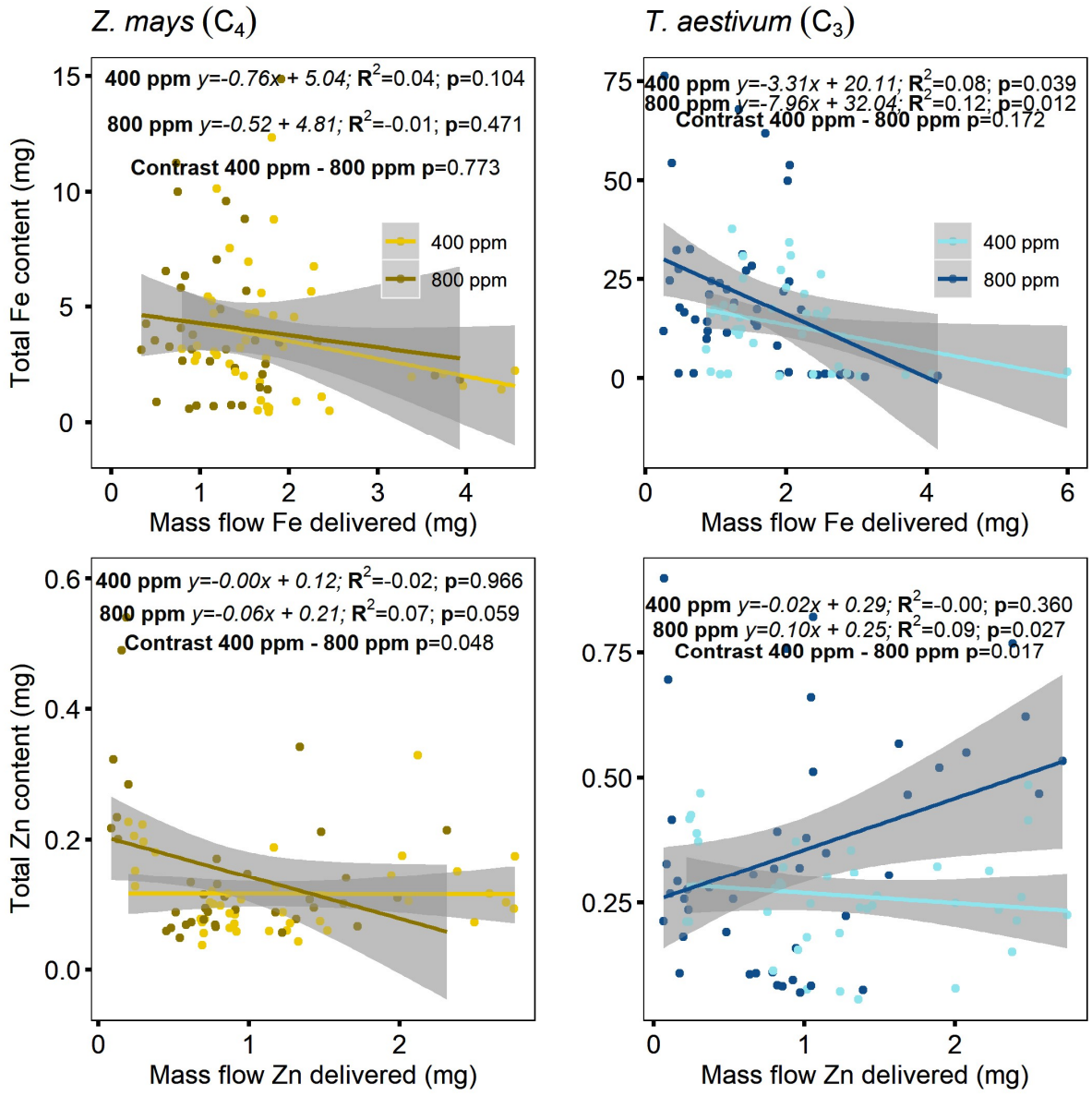
Appendix 7 Variation in total tissue contents of Ca, Cu and Mn in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Appendix 1.



Appendix 8 Variation in total tissue contents of Fe and Zn in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Appendix 1.



Appendix 9 The relationship between transpiration-driven mass flow delivery of Ca, Cu and Mn, and total tissues contents of Ca, Cu and Mn, in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. The intercept, slope, R^2 and p-values of the lines are displayed, as well as the p-value of the contrast between the 400 and 800 ppm line.



2343

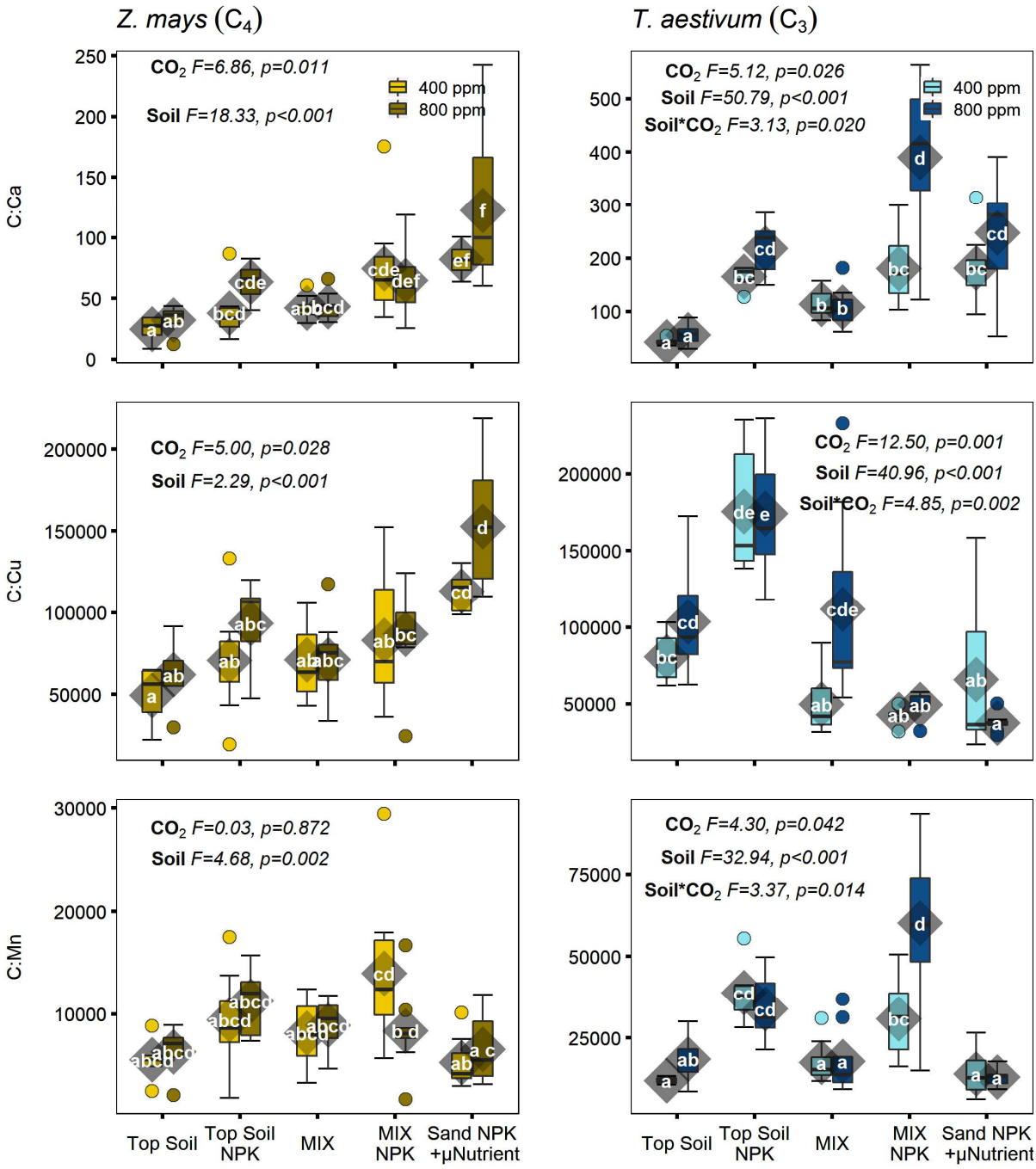
2344 **Appendix 10** The relationship between transpiration-driven mass flow delivery of Fe and Zn,

2345 and total tissues contents of Fe and Zn, in maize and wheat plants, grown in five soils with

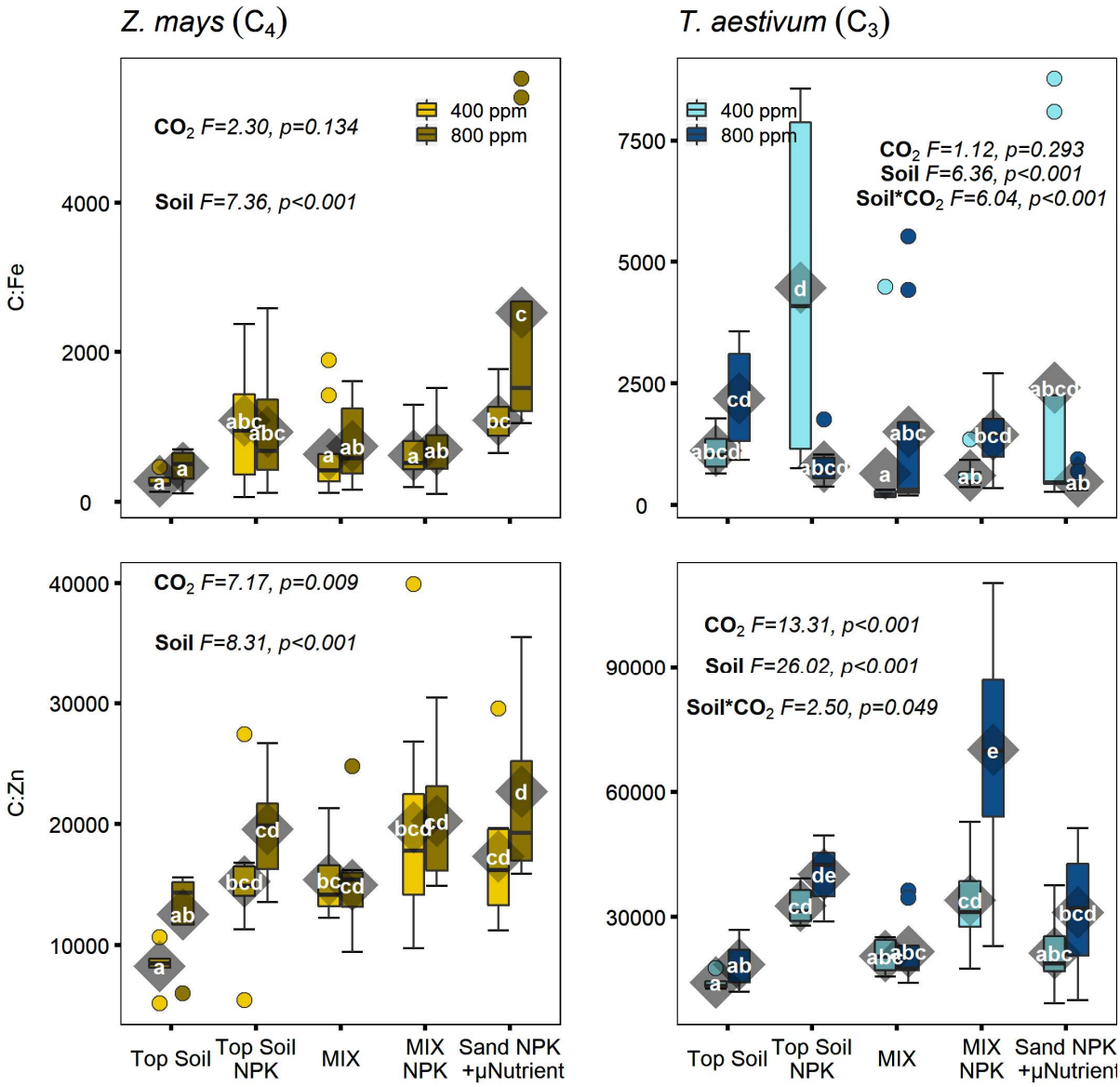
2346 differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Appendix

2347 9.

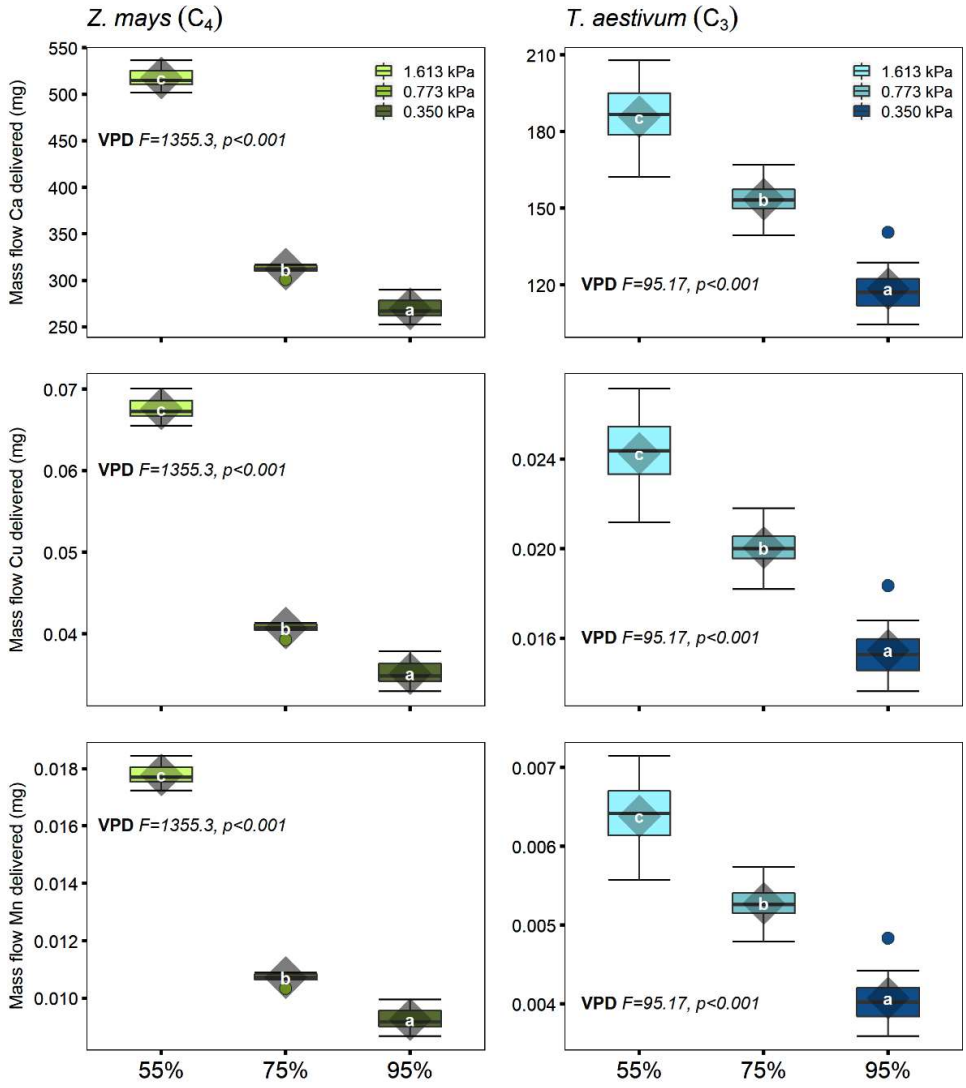
2348



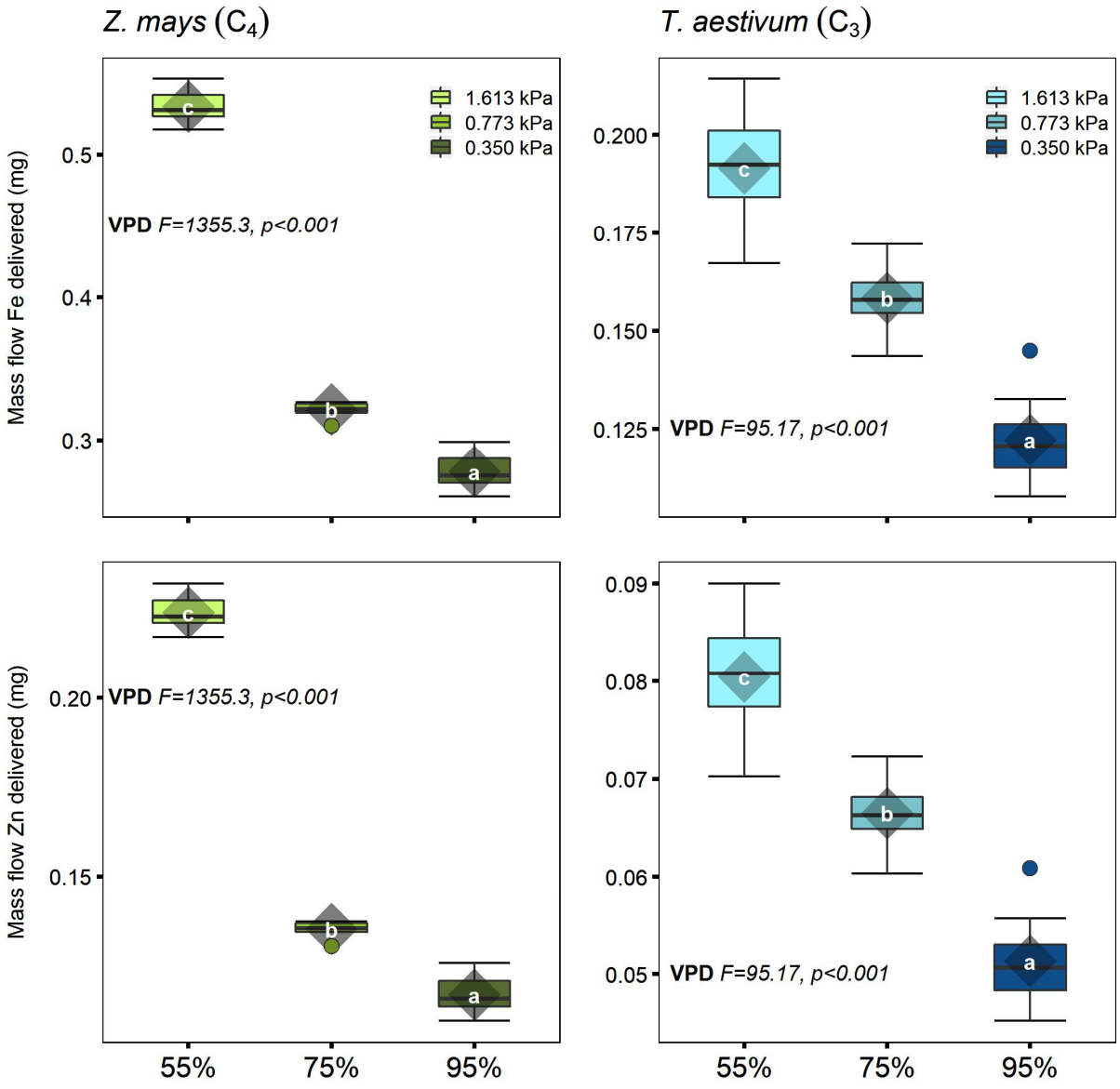
Appendix 11 Variation in the C:Ca, C:Cu and C:Mn ratios in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Appendix 1.



Appendix 12 Variation in the C:Fe and C:Zn ratios in maize and wheat plants, grown in five soils with differing nutrient availabilities at two CO_2 levels, 400 and 800 ppm. Statistics as in Appendix 1.



Appendix 13 Variation in transpiration-driven mass flow delivery of Ca, Cu and Mn in maize and wheat plants, grown in Soil, at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. The boxes and horizontal lines represent the first and the third quartiles and the medians, respectively. The whiskers represent 1.5 × the interquartile range and outliers above/below are shown as points. The diamonds represent the mean values. The F- and p-values of the main effects and interaction (when significant/present) from the analysis of variance done on the linear models fitted are displayed. Lower case letters indicate the significant differences between treatments as determined by Tukey's post-hoc comparisons. Corresponding air relative humidity (in %) at 25°C shown on the x-axis.



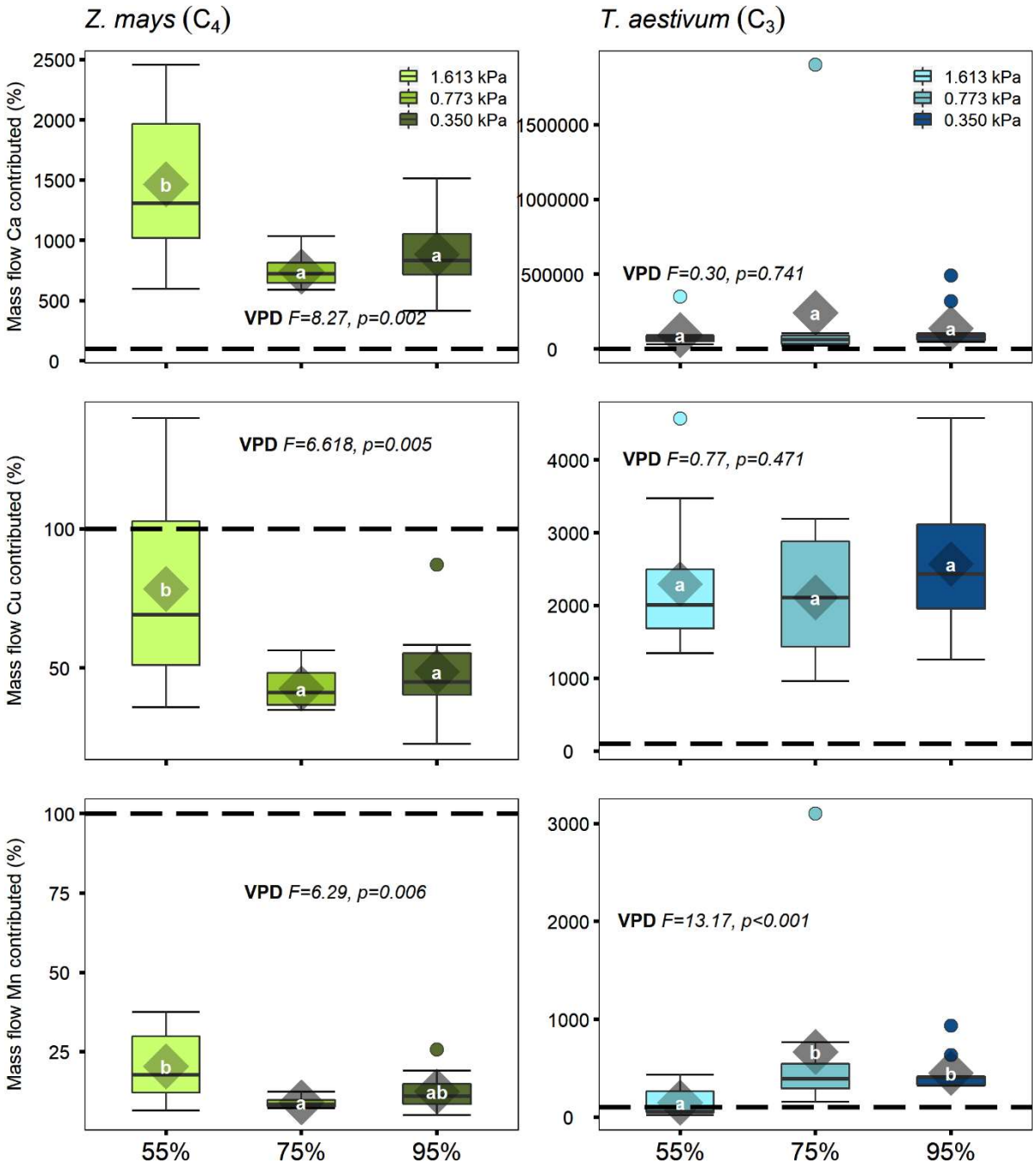
2373

2374 **Appendix 14** Variation in transpiration-driven mass flow delivery of Fe and Zn in maize and

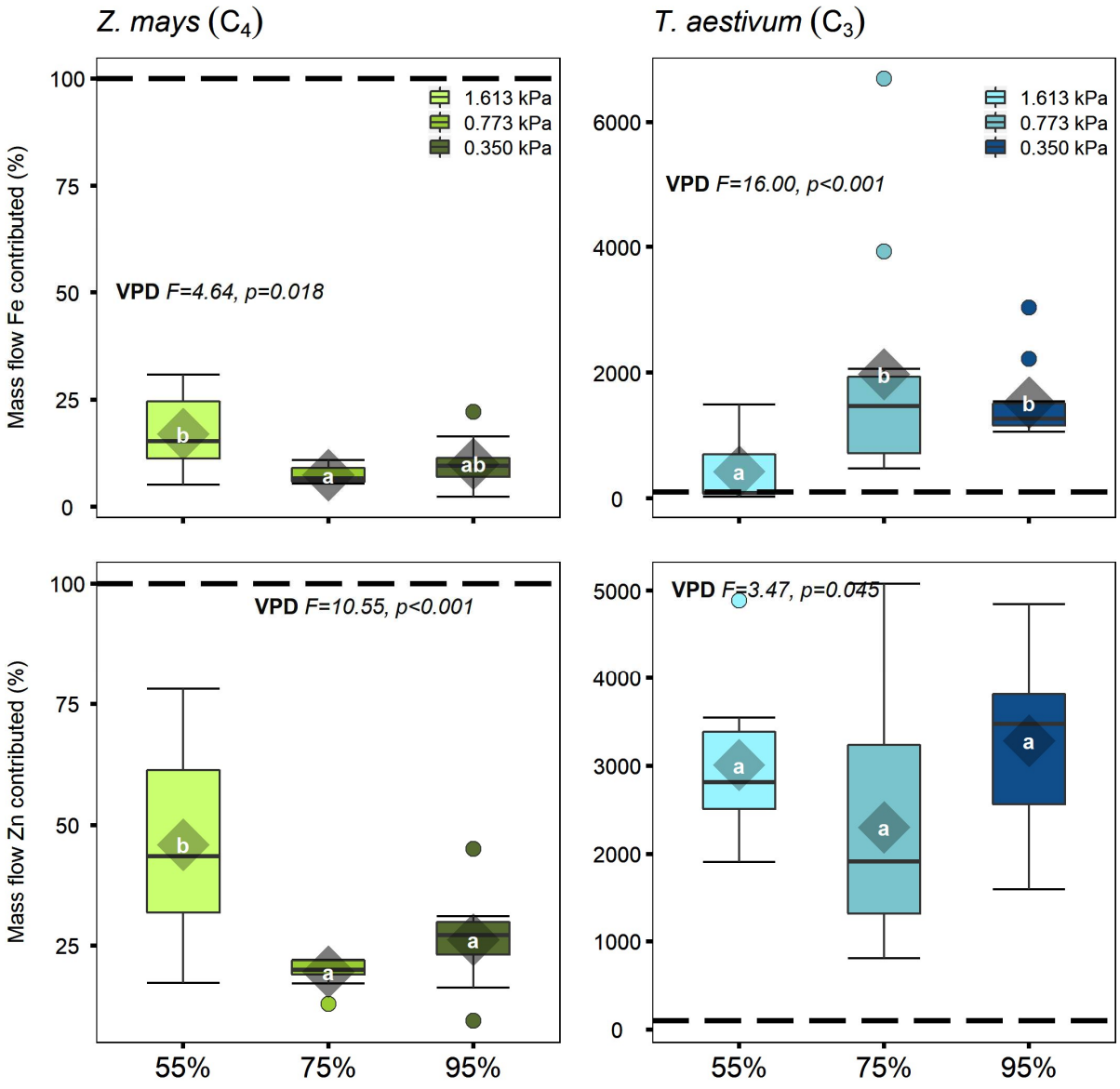
2375 wheat plants, grown in Soil, at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics

2376 as in Appendix 1. Corresponding air relative humidity (in %) at 25°C shown on the x-axis.

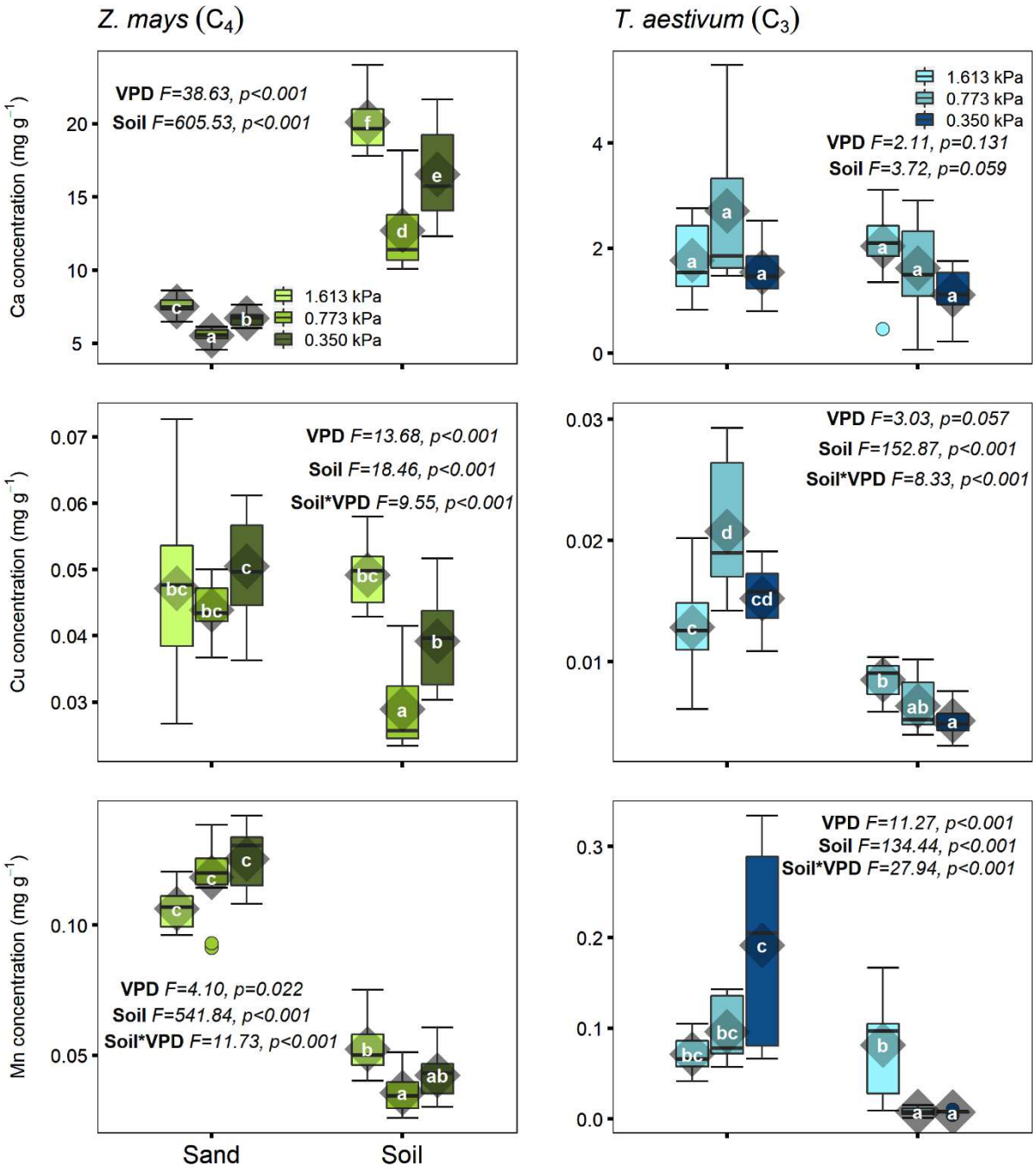
2377



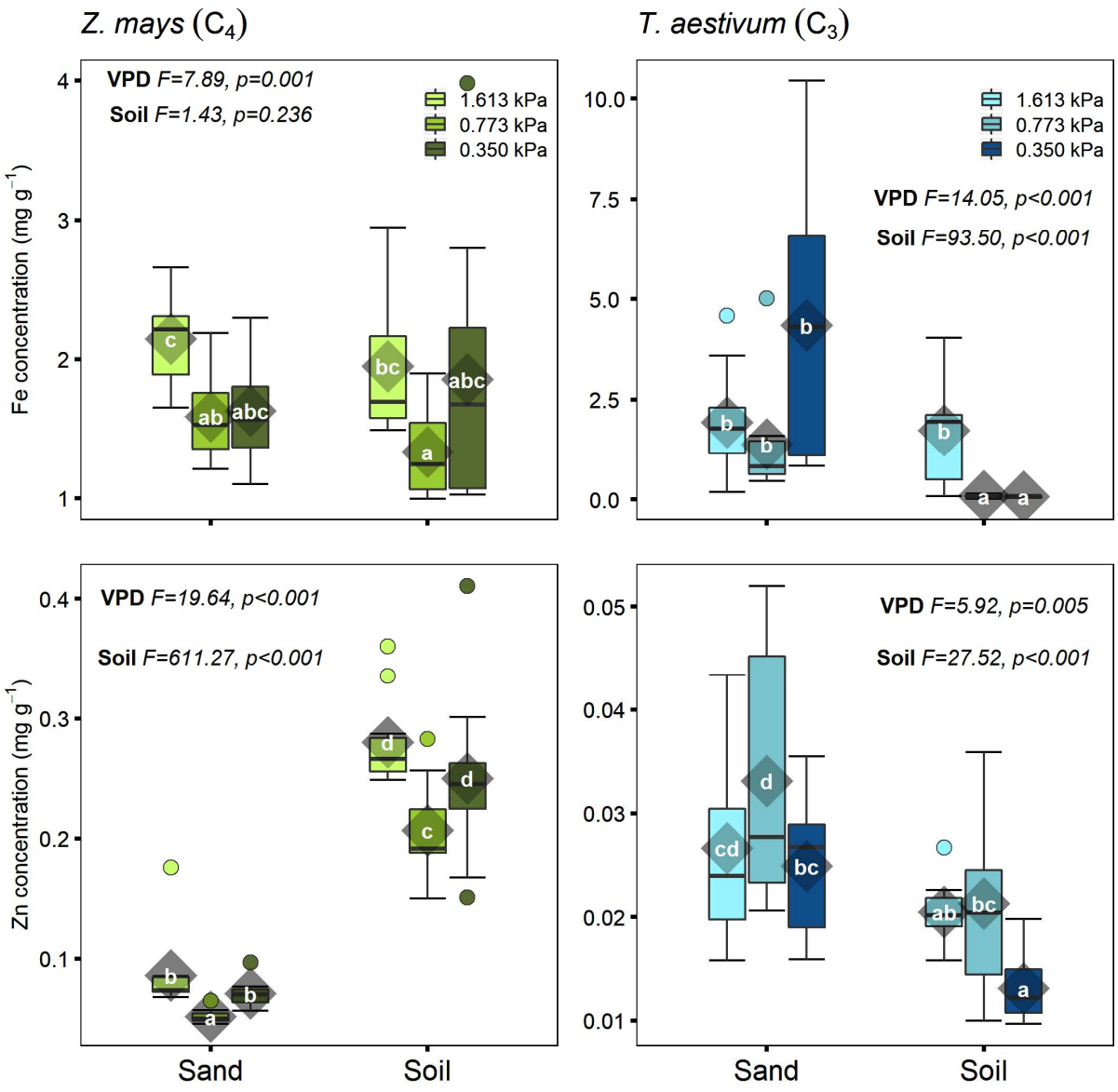
Appendix 15 Variation in transpiration-driven mass flow contribution of Ca, Cu and Mn to tissue content in maize and wheat plants, grown in Soil, at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. The dashed line represents 100% contribution to tissue content. Statistics as in Appendix 1. Corresponding air relative humidity (in %) at 25°C shown on the x-axis.



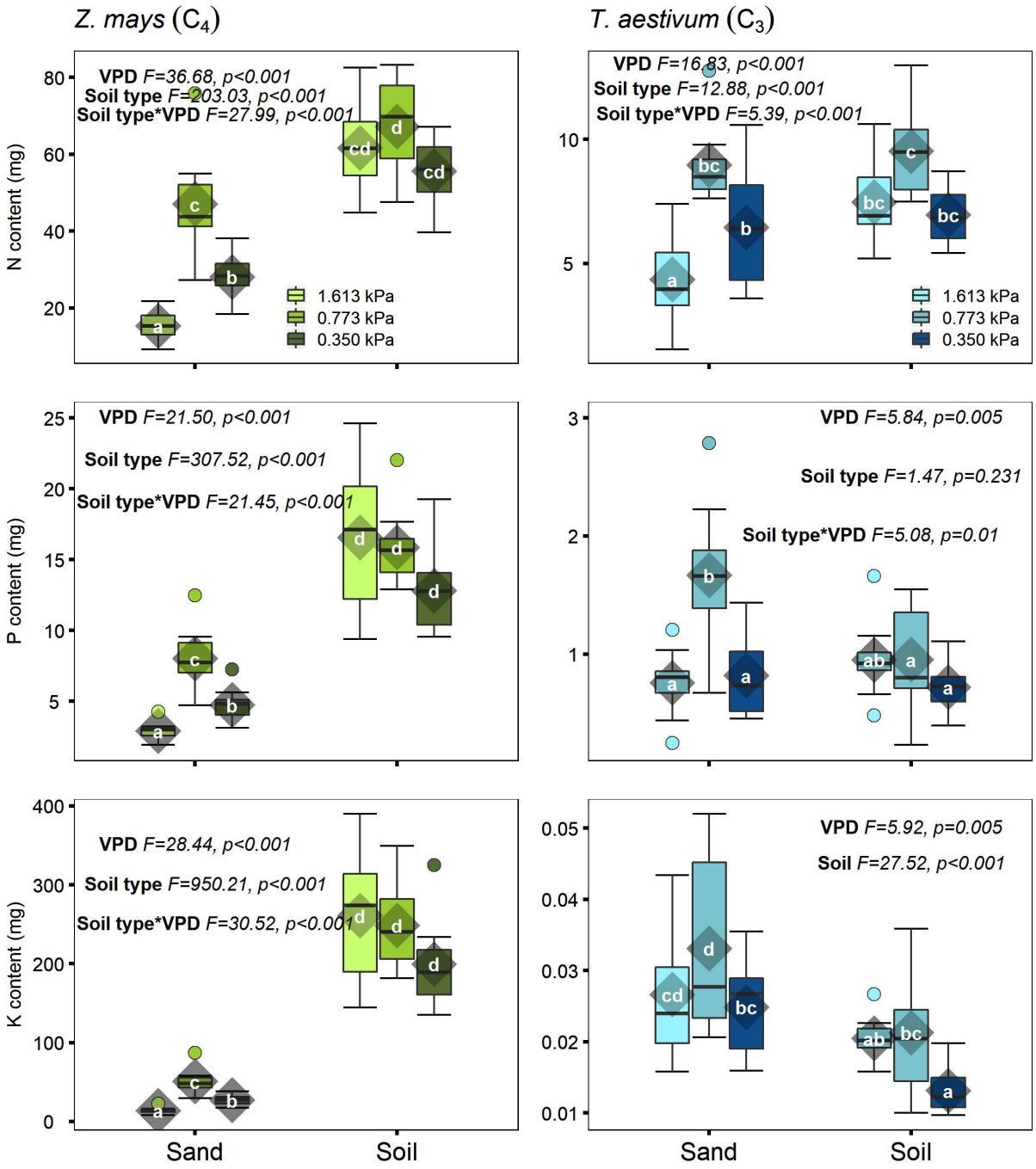
Appendix 16 Variation in transpiration-driven mass flow contribution of Fe and Zn to tissue content in maize and wheat plants, grown in Soil, at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. The dashed line represents 100% contribution to tissue content. Statistics as in Appendix 1. Corresponding air relative humidity (in %) at 25°C shown on the x-axis.



Appendix 17 Variation in total tissue concentrations of Ca, Cu and Mn in maize and wheat plants, grown in Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics as in Appendix 1.



Appendix 18 Variation in total tissue concentrations of Fe and Zn in maize and wheat plants, grown in Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics as in Appendix 1.



2401

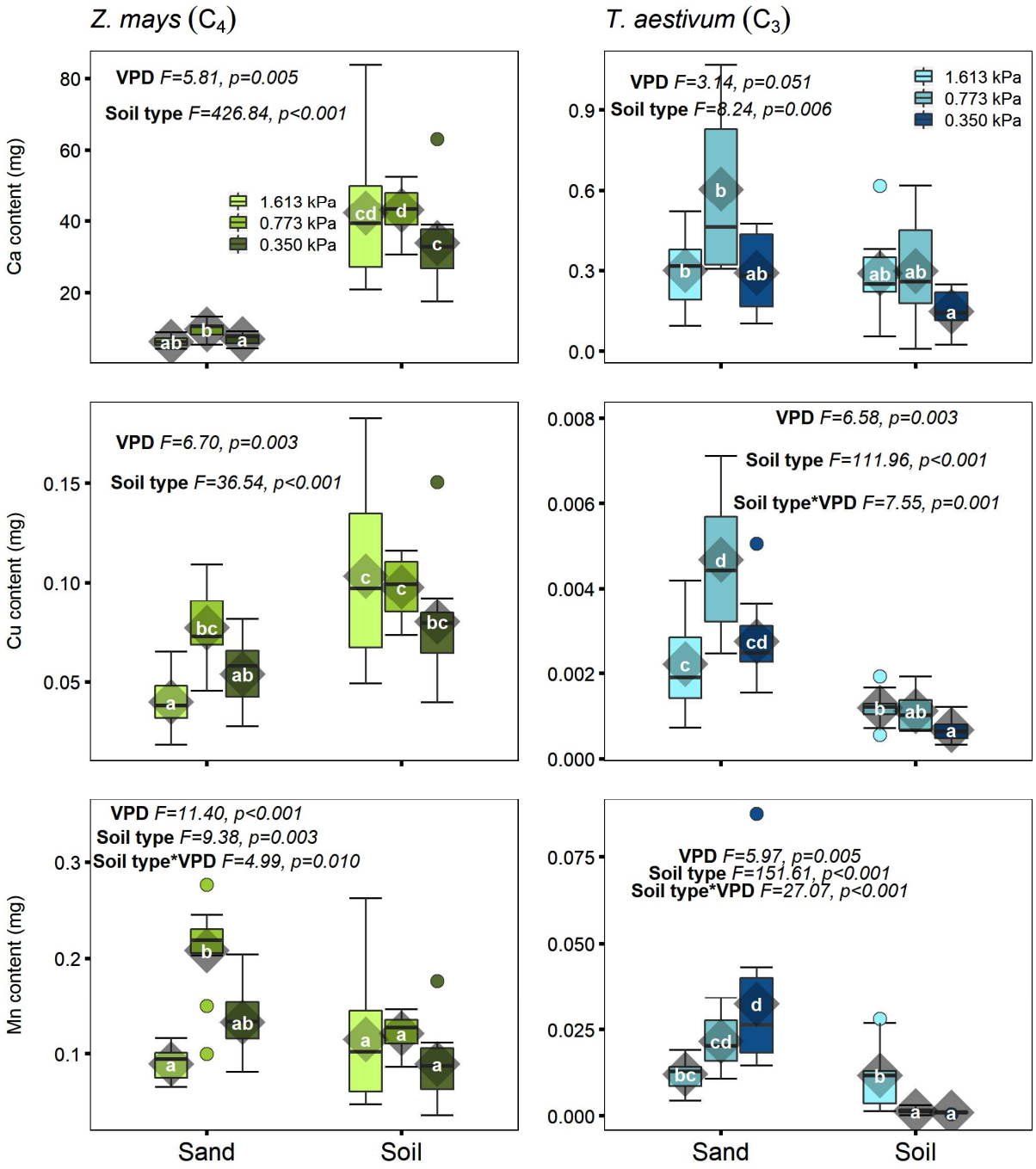
2402 **Appendix 19** Variation in total tissue contents of N, P and K in maize and wheat plants, grown

2403 in Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics as in

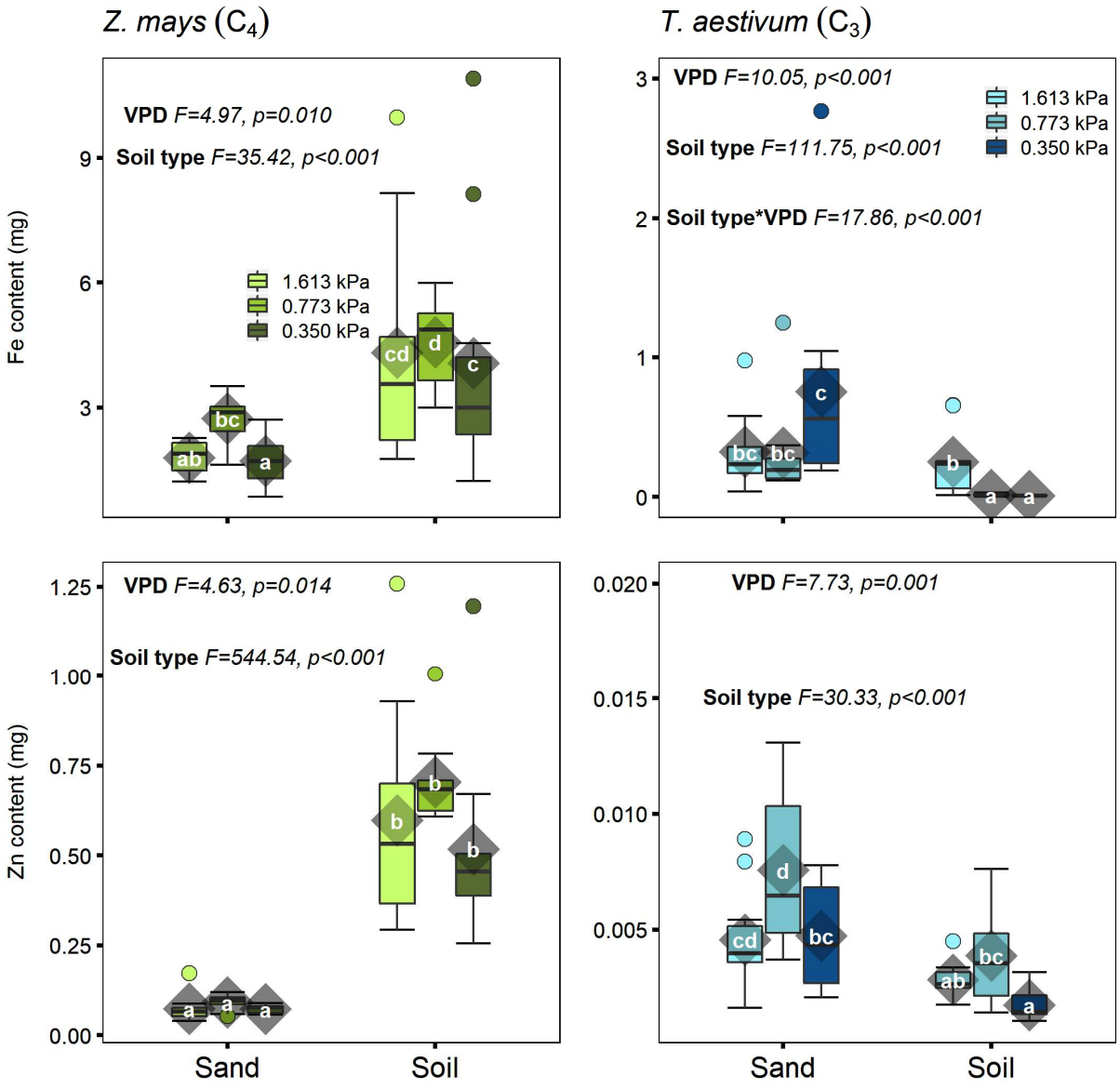
2404 Appendix 1.

2405

2406



Appendix 20 Variation in total tissue contents of Ca, Cu and Mn in maize and wheat plants, grown in Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics as in Appendix 1.



Appendix 21 Variation in total tissue contents of Fe and Zn in maize and wheat plants, grown in Soil and in Sand at three VPD levels, 1.613 kPa, 0.773 kPa and 0.350 kPa. Statistics as in Appendix 1.